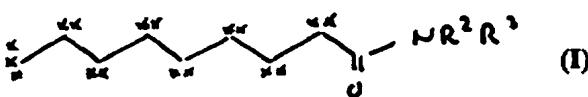
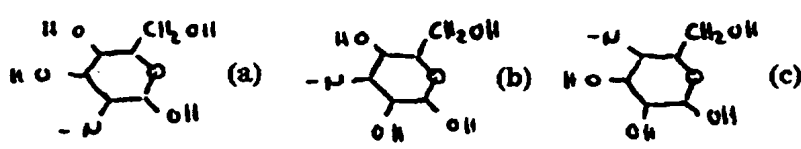




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(54) Title: <b>AMIDES OF MONO AND BICARBOXYLIC ACIDS WITH AMINO ACIDS OR GLYCOSAMINES, SELECTIVELY ACTIVE ON THE CANNABINOID PERIPHERAL RECEPTOR</b>			
 <p style="text-align: right;">(I)</p>			
 <p style="text-align: center;">(a)                      (b)                      (c)</p>			
(57) Abstract			
<p>The vast number of theoretically conceivable compounds resulting from the combination of all claimed substituents in formula (I) precludes a comprehensive search. For economical reasons the search has been limited to the following compounds: R<sub>1</sub> is a hydrocarbon radical unsubstituted or substituted by only one OH-group and having a linear chain of at least 8 carbons atoms(A); the NR<sub>2</sub>R<sub>3</sub> group is either a linear alpha-, beta-, or gamma-, amino acid (e.g. amino acids like proline are not included), or a glycosamine residue. Despite the above limitation the search revealed too many relevant compounds. Therefore the search was further limited to compounds with NR<sub>2</sub>R<sub>3</sub> = Glycine, beta-Alanine, gamma-amino-butyric acid, Serine- or Cysteine derivatives, or glycosamine of the following structures: (a), (b), (c). Still too many pertinent compounds were retrieved which can not all be cited in the search report. The documents cited are only an arbitrary selection and the search report has to be regarded as incomplete.</p>			

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- 1 -

**AMIDES OF MONO AND BICARBOXYLIC ACIDS WITH AMINO ACIDS OR GLYCOSAMINES,  
SELECTIVELY ACTIVE ON THE CANNABINOID PERIPHERAL RECEPTOR****FIELD OF THE INVENTION**

The present invention refers to new amides of mono and bicarboxylic  
5 acids with aminoalcohols, therapeutically active in the treatment of  
pathologies connected with the modulation of the peripheral receptor  
for cannabinoids.

**STATE OF THE ART**

Cannabinoids are a specific class of psycho-active compounds present  
10 in hemp (*Cannabis sativa*), comprising more than 60 different  
molecules, the most important of which are: cannabinol, cannabidiol  
and many isomers of tetrahydrocannabinol. The therapeutic activity of  
*Cannabis* is known since the old chinese dynasties which used it  
already 5000 years ago in the treatment of asthma, migraine and some  
15 gynecological disorders. The use was confirmed along the years and in  
1850 the *Cannabis* extracts where entered the American Pharmacopoeia  
and maintained up to 1947.

Cannabinoids are responsible of many effect on different systems  
and/or organs; the most relevant effects are on the central nervous  
20 system and on the cardiovascular system. In fact, they affect mood,  
memory, motory coordination and cognition, increase heart rate and  
modify the systemic arterious pressure. It is also known that  
cannabinoids are capable of lowering the intraocular pressure and  
produce effects on the respiratory and endocrin system (L.E.  
25 Hollister, Health Aspects of Cannabis, Pharmacological Reviews, 38, 1-  
20, 1986). More recently also their capacity of suppressing the  
cellular and humoral immune response and their antiinflammatory

- 2 -

properties were described (A. W. Wirth et al., Antiinflammatory properties of Cannabichromene, Life Science 26, 1991-95, 1980).

However, the use of Cannabis in therapy is debated, because of its relevant psychoactive effects, which gives addiction and dependence, and its various side effects which up to now are not completely known (L.E. Hollister, 1986, cited above).

In spite of the large use made along the centuries, the mechanism of action of cannabinoids was for long unknown. Only in 1990 Matsuda et al. could identify and clone a receptor for cannabinoids which was discovered to be a member of the super-family of the G-protein-coupled receptors; CB1 is coupled to G1 to inhibit adenylate cyclase activity and to a pertussis-sensitive G protein to regulate  $Ca^{2+}$  currents. Such receptor is mainly localised in the brain, in cell-lines of nervous origin and only to a limited extent at peripheral level; therefore, depending on its location, it was named Central Receptor (CB1) (Matsuda et al., Structure of a cannabinoid receptor and functional expression of the cloned cDNA, Nature 346:561-564, 1990). The discovery of a receptor led to the assumption that the existence of a specific endogenous ligand. Other studies permitted to isolate in the pig brain a substance capable of producing an antagonist effect, i.e. capable of competitively binding to the cannabinoid central receptor. The structural study and the comparative tests with the synthetic product permitted to identify such substance, which resulted to be an amidic derivative of arachidonic acid, and more particularly the arachidonylethanolamide, later named anandamide. The pharmacological characterization of such molecule showed that anandamide possesses an activity profile similar, although less potent, than 9-THC

- 3 -

(tetrahydrocannabinol with a double bond in position 9), and is able to mimic its psychoactive effects. Such results brought to the conclusion that anandamide is the endogenous ligand of the central receptor for cannabinoids (C. C. Felder et al. Anandamide, an  
5 endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction, PNAS. 90, 7656-7660, 1993; P.B. Smith et al. The pharmacological Activity of Anandamide, a Putative Endogenous Cannabinoid, in Mice, J. PET. 270, 219-227, 1994).

10 Following studies permitted to identify substances capable of binding to the receptor CB1, which were grouped in a class of amidic compounds named anandamides by the authors (Hanus L. et al. Two New Unsaturated Fatty Acid Ethanolamides in Brain That Bind to the Cannabinoid Receptor, J. Med. Chem., 36, 3032-3034, 1993). The discovery that the  
15 ethanolamide of arachidonic acid, but not the ethanolamide of other acids which are biologically important and anyway endogenously present at the cerebral level (such as palmitic acid), is capable of functionally activating CB1 central receptor, brought about the subsequent identification of other amides of ethanolamine with highly  
20 unsaturated fatty acids which have an affinity to CB1 receptor.

Its peculiar distribution led to assume the existence of differentiated receptor sites. In fact, a second different receptor for cannabinoids was cloned, named Peripheral Receptors (CX5 or CB2). Since such receptor was identified in the spleen and  
25 macrophages/monocytes, being on the other hand absent at the central level, it is considered that this receptor mediates the non psychoactive effects of the cannabinoids (S. Munro et al., Molecular

- 4 -

characterisation of a peripheral receptor for cannabinoids, Nature, 365, 61-65, 1993). In this connection there are some evidences of the capacity of  $\Delta^9$ -THC to induce immunosuppressive effects. Recent experimental studies demonstrated that  $\Delta^9$ -THC is capable of

5 influencing the function of macrophage. Exposition to  $\Delta^9$ -THC lowers the cytolytic action of activated macrophages, measured as synthesis, release and cytotoxicity of TNF- $\alpha$ . Anyhow, since the macrophages release various molecules having a cytolytic potential, other than TNF- $\alpha$ , it is considered that they can represent a target for  $\Delta^9$ -THC (K.

10 Fischer-Stenger et al.,  $\Delta^9$ -tetrahydrocannabinol Inhibition of Tumor necrosis Factor- $\alpha$ : Suppression of Post-translational Events, J. PET, 267, 1558-1565, 1993). All these evidences and the preferential massive localisation of CB2 receptor in the immuno system confirm that such receptor plays a specific role in mediating the immune and

15 antiinflammatory response to stimuli of different nature, included the bacterial and the viral ones.

It was also demonstrated that anandamide, endogenous ligand for the CB1 central receptor, is capable of binding to the CB2 receptor with an affinity which is 30 folds inferior to that of the central

20 receptor; this probably implies the existence of a different endogenous ligand for such receptor, up to now still unknown (L. L. Iversen, Medical uses of marijuana?, Nature, 365, 12-13, 1993).

As already mentioned, the therapeutical use of cannabinoids as analgesics, antiemetics, anticonvulsivants, antispastics, antiglaucoma

25 and, more recently, antiinflammatory, is limited by the presence of undesired side effects, or psychoactive effects, and by the outcome of addiction and pharmacological tolerance (W.L. Dewey, Cannabinoid

- 5 -

Pharmacology, Pharmacological Reviews, 38, 151-178, 1986).

Recently some compounds were prepared, capable of acting as agonists on both the cannabinoids receptors: for example it is known the use of derivatives of dihydropyrrole-(1,2,3-d,e)-1,4-benzoxazine in the treatment of glaucoma (US 5 112 820, Derwent abstract) and it is also known the use of derivatives of 1,5-diphenyl-pyrazole as immunomodulators or psychotropic agents in the treatment of various neuropathologies, migraine, epilepsy, glaucoma ecc. (European Patent Application EP 576 357, Derwent abstract).

10 However, such compounds, being active on both CB1 central and CB2 peripheral receptor, can lead to the outcome of serious psychoactive effects, a part from addiction and dependence.

In the light of the fact that the cannabinoids act through a receptorial mechanism and especially on receptors capable of mediating different functional effects, and also in view of the low homology between the Peripheral and Central Receptors, it is evident the importance of developing a class of drugs selectively active on the receptor sub-type and not indiscriminately on both the receptors such as the natural or synthetic cannabinoids.

20 The search on the pharmacological effects mediated by cannabinoids receptors shows that the non-psychoactive effects of Cannabis derivatives are mediated by CB2 peripheral receptor. Furthermore, receptor CB2 localisation confirms that such non-psychoactive effects, i.e. the effects on the immune system and the antiinflammatory, myorelaxant and antinociceptive effects, as well as the influence on the pressure systems, are mediated by such receptor.

It is therefore extremely important to obtain compounds which are

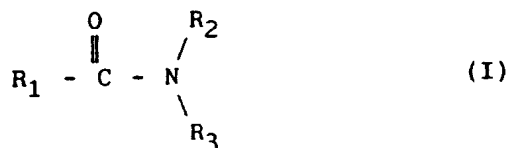
- 6 -

capable of selectively acting on the peripheral receptor for cannabinoids and, therefore, on the pathologies connected with the modulation of such receptor, excluding the psychoactive effects at central level and the relevant side effects related to such action, such as addiction and dependence.

## SUMMARY OF THE INVENTION

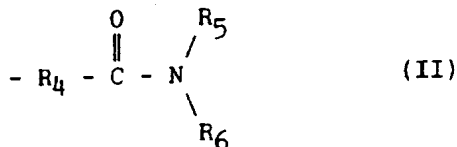
The Applicant has now found a new class of amidic derivatives of aliphatic mono and dicarboxylic acids with aminoalcohols, able to bind selectively to the cannabinoid peripheral receptor CB2 and to activate functionally the same.

The new amidic derivatives according to the present invention have formula (I):



wherein  $\text{R}_1$  is:

- 1) a linear or branched hydrocarbon radical having from 9 to 23 carbon atoms, preferably from 11 to 17, saturated or presenting one double bond, optionally substituted with one or more -OH groups;
- 2) a group of formula (II):

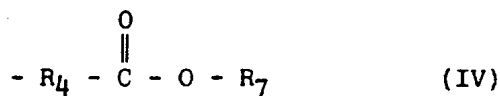


wherein  $\text{R}_4$  is a linear hydrocarbon radical, saturated or containing one double bond, comprising from 8 to 22 carbon atoms, preferably from 10 to 16, optionally substituted with one or more linear or branched alkyl groups  $\text{C}_1$ - $\text{C}_8$  and/or with one or more -OH groups;



- 7 -

3) a group of formula (IV):



wherein  $R_4$  has the meanings described above and  $R_7$  is -H or a linear or branched alkyl group comprising from 1 to 20 carbon atoms.

In formula (I) the residue of formula (III):



can represent the aminic residue of an aminoacid (A) or of a  
5 glycosamine (B), and in particular:

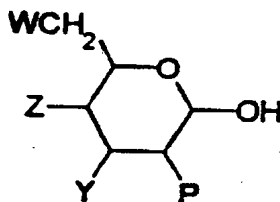
A) the residue of formula (III) is the residue of an optically active or non active aminoacid, wherein  $R_2$ , together with the nitrogen atom to which it is bound, forms an  $\alpha$ -aminoacid, optionally presenting an aliphatic or arylaliphatic lateral chain optionally substituted  
10 with -OH, -OPO<sub>3</sub>H<sub>2</sub>, -O-PO<sub>2</sub>H-O-CH<sub>2</sub>-CH(OH)-CH<sub>2</sub>-OH, -SH or S-CH<sub>3</sub>; or it forms, together with the nitrogen atom to which it is bound, a  $\beta$ - or  $\gamma$ -aminoacid presenting an aliphatic or arylaliphatic lateral chain; the acid group of such aminoacid being optionally methylated or ethylated;

15  $R_3$  is -H or -CH<sub>3</sub>;

when  $R_1$  belongs to class (1) and  $R_2$  forms, together with the N atom to which it is bound, the aminoacylic residue of alanine,  $\beta$ -alanine or glycine.  $R_3$  is different from -CH<sub>3</sub>;

B) in the residue of formula (III),  $R_2$  forms, with the nitrogen atom  
20 to which it is bound, the residue of a glycosamine having formula

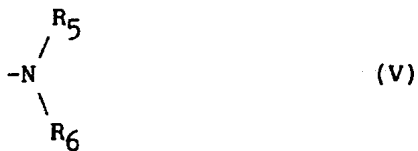
- 8 -



wherein one of the groups P, Y and Z is  $\begin{array}{c} -N-CO-R_1 \\ | \\ R_3 \end{array}$  and the

other groups can be -H or -OH,  $R_1$  being as above defined and  $R_3$  being -H or -CH<sub>3</sub>.

In formula (II) the residue of formula (V):



5 can be the same as (III), defined above, with  $R_5$  and  $R_6$  equal or different from  $R_2$  and  $R_3$ .

The residues of formulae (III) and (V), belonging both to classes (A) and (B), can be optionally homogeneously substituted on  $R_2$ ,  $R_3$ ,  $R_5$  and/or  $R_6$  with at least one pharmaceutically acceptable radical  
 10 capable of increasing the solubility of the compounds of the present invention in water, alcohols and suitable organic solvents. Said pharmaceutically acceptable radical is preferably selected from the group consisting of acyls (i.e. acetyl and benzoyl), emiacyls of dicarboxylic acids (i.e. succinic and glutaric acid), amino-acyles,  
 15 dialkylamino-acyls, alkyl-sulphonates (i.e. metansulphonate and p-toluen-sulphonate), alkyls (i.e. methyl and ethyl), O-phosphates and O-sulphates.

A further object of the present invention is the use of the described derivatives in the treatment of pathologies of mammals connected with

- 9 -

the modulation of the cannabinoid peripheral receptor.

Other objects of the present invention are the process for the preparation of the above described new compounds and the pharmaceutical compositions containing therapeutically active quantities of such amidic derivatives and of their more soluble and/or slow release form, for the therapeutic treatment of pathologies connected with the modulation of the cannabinoid peripheral receptor.

#### DESCRIPTION OF THE DRAWINGS

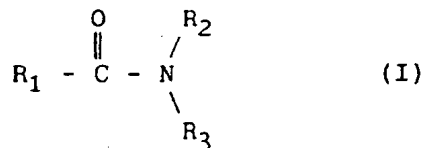
Figure 1 illustrates the specific hybridization of cannabinoid peripheral receptor amplified by Polymerase Chain Reaction (PCR) in A) rat spleen, B) rat peritoneal mast cell cultures and C) RBL-2H3 cell cultures.

Figure 2 illustrates the specific mRNA-CB2 cannabinoid peripheral receptor detected by in situ hybridisation in Cerebellar granule cells (Fig. 2A) and Cerebellum (Fig. 2B).

#### DETAILED DESCRIPTION OF THE INVENTION

The advantages and characteristics of the new class of amides of mono or bicarboxylic acids with aminoalcohols, selectively active on the cannabinoid peripheral receptor, the process for their preparation, their pharmaceutical use and the pharmaceutical compositions containing them, according to the present invention, will be better illustrated in view of the following detailed description.

The amides of mono and bicarboxylic acids with aminoalcohols according to the present invention have formula (I):



- 9a -

When  $R_1$  belongs to class (1), it forms, with the adjacent carbonyl, the acyl group of a monocarboxylic acid, preferably lauric, myristic, palmitic, stearic, palmitoleic, oleic acid and their homologues substituted with hydroxyl groups, as for example -hydroxypalmitic  
5 acid.

When  $R_1$  belongs to classes (2) and (3),  $R_4$  forms, together with the two adjacent carbonyls, the acyl group of a bicarboxylic acid, preferably traumatic acid.

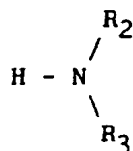
- 10 -

As regards the aminic moiety of formula (I), when the residue of formula (III) belongs to class (A), such aminoacid is preferably glycine,  $\beta$ -alanine,  $\gamma$ -amino-butyric acid, serine and cysteine.

Are known in the state of the art the compounds of formula (1) wherein  
 5  $R_1$  belongs to class (1) and  $R_2$  forms, together with the nitrogen atom to which it is bound, the aminoacylic residue of alanine,  $\beta$ -alanine and glycine, and  $R_3 = CH_3$ .

When the residue of formula (III) belongs to class (B), such glycosamine is preferably D-Glucosamine, L-Acosamine, D-Mannosamine,  
 10 D-Galactosamine.

The process for the preparation of said amides of formula (I), according to the invention, involves the reaction of an activated form of the acid  $R_1$ -COOH, of formula  $R_1$ -CO-X, with an amine of formula



in an aqueous basic medium or in organic solvents in the presence of  
 15 bases, under stirring at a temperature ranging from  $-20^\circ$  to  $60^\circ\text{C}$ , and preferably from  $-10^\circ$  to  $20^\circ\text{C}$ , for a period ranging from 10 minutes to 24 hours, and preferably from 1 to 3 hours.

After the optional acidification of the reaction medium, the desired product is isolated and purified by conventional techniques.

20 Said activated form of the acid can be an acyl halide, a succinimide-ester, an acylisourea, a p- $\text{NO}_2$ -phenylester or a methylester; said base is preferably  $\text{K}_2\text{CO}_3$ , KOH,  $\text{Et}_3\text{N}$  or N-methyl-morpholine.

The following examples of preparation of the amides of mono and bicarboxylic acids according to the present invention are reported for

- 11 -

illustrative but not limitative purposes.

**EXAMPLE 1: Preparation of N-palmitoyl-L-serine**

1.58 g of L-serine (15 mmol) are solubilized at 4°C in 60 ml of K<sub>2</sub>CO<sub>3</sub> 1M. The solution is slowly added, dropwise under stirring, over a  
5 period of 30 minutes, with 2.75 g of palmitoyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight, thereafter is acidified with hydrochloric acid 6N. The precipitated crude product is separated by filtration, dried under vacuum and crystallized a first time from 30 ml of ter-butyl-methyl-ether and  
10 thereafter from 30 ml of cold methanol. The crystallized product is separated by filtration, washed twice with 5 ml methanol and dried under high vacuum.

The reaction reaction yield is about 89%.

The physico-chemical properties of N-palmitoyl-L-serine are as  
15 follows:

-physical state:	white crystalline powder
-molecular formula:	C <sub>19</sub> H <sub>37</sub> NO <sub>4</sub>
-molecular weight:	343.51
-elemental analysis:	C= 66.43%. H= 10.86% N= 4.08% O= 18.63%
20 -solubility in org. sol.:	>10 mg/ml in DMSO >10 mg/ml in ethanol
-water solubility:	slightly soluble
-melting point:	97-99°C
-TLC:	eluent: chloroform/methanol/water/ 25 NH <sub>3</sub> (28%) 80:25:2:1 R <sub>f</sub> = 0.15

**EXAMPLE 2: Preparation of N-lauroyl-L-serine**

1.58 g of L-serine (15 mmol) are solubilized at 4°C in 40 ml of KOH

- 12 -

1N. The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.19 g of lauroyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight, thereafter is acidified with hydrochloric acid 6N and extracted three  
5 times with 20 ml ethyle acetate; the organic phases are collected, dried with anhydrous sodium sulphate and evaporated under vacuum. The crude product is crystallized from 30 ml of cold acetonitrile. The crystallized product is separated by filtration, washed twice with 5 ml acetonitrile and dried under high vacuum.

10 The reaction yield is about 85%.

The physico-chemical properties of N-lauroyl-L-serine are as follows:

- physical state: white crystalline powder
- molecular formula:  $C_{15}H_{29}NO_4$
- molecular weight: 287.40
- 15 -elemental analysis: C= 62.69% H= 10.17% N= 4.87% O= 22.27%
- solubility in org. sol.: >10 mg/ml in DMSO  
>10 mg/ml in ethanol
- water solubility: slightly soluble  
(>10 mg/ml as sodium salt)
- 20 -melting point: 120-122°C
- TLC: eluent: toluene/ethanol/acetic acid  
65:30:5 Rf = 0.46

#### EXAMPLE 3: Preparation of N-oleoyl-L-serine

1.58 g of L-serine (15 mmol) are solubilized at 4°C in 60 ml of  $K_2CO_3$   
25 1M. The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 3.01 g of oleoyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight,

- 13 -

thereafter is acidified with hydrochloric acid 6N. The precipitated crude product is separated by filtration, dried under vacuum and purified by silica-gel column chromatography using as eluent a mixture of chloroform/methanol/water/acetic acid 90:10:0.5:0.1. The fractions  
5 of eluate containing the product are collected, evaporated to dryness and the residue is dried under high vacuum.

The reaction yield is about 88%.

The physico-chemical properties of N-oleoyl-L-serine are as follows:

- physical state: white amorphous powder
- 10 -molecular formula:  $C_{21}H_{39}NO_4$
- molecular weight: 369.54
- elemental analysis: C= 68.26% H= 10.64% N= 3.79% O= 17.32%
- solubility in org. sol.: >10 mg/ml in DMSO
- water solubility: slightly soluble
- 15 -melting point: -
- TLC: eluent: chloroform/methanol/water/  
 $NH_3$  (28%) 80:25:2:1  $R_f$  = 0.14

#### EXAMPLE 4: Preparation of N-palmitoleyl-L-serine

1.58 g of L-serine (15 mmol) are solubilized at 4°C in 60 ml of  $K_2CO_3$   
20 1M. The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.73 g of palmitoyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight, thereafter is acidified with hydrochloric acid 6N and extracted three times with 20 ml ethyle acetate; the organic phases are washed twice  
25 with 15 ml of water, collected and evaporated under vacuum. The crude product is purified by preparative silica-gel column chromatography, using as eluent a mixture of chloroform/methanol/water/acetic acid



- 14 -

90:10:0.5:0.1. The eluate fractions containing the product are collected, evaporated to dryness and the residue is dried under high vacuum.

The reaction yield is about 87%.

5 The physico-chemical properties of N-palmitoleyl-L-serine are as follows:

-physical state: white amorphous powder

-molecular formula:  $C_{19}H_{35}NO_4$

-molecular weight: 341.49

10 -elemental analysis: C= 66.83% H= 10.33% N= 4.10% O= 18.74%

-solubility in org. sol.: >10 mg/ml in DMSO

-water solubility: slightly soluble

-melting point: -

-TLC: eluent: toluene/ethanol/acetic acid

15 65:30:5 Rf: 0.45

#### EXAMPLE 5: Preparation of N-lauroyl-L-serine methylester

2.33 g of L-serine methylester hydrochloride (15 mmol) are solubilized at 4°C in 40 ml of a mixture tetrahydrofuran/water 9:1 with 4.05 g of triethylamine (40 mmol). The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.19 g of lauroyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C for 2 h and at room temperature for a night and evaporated to dryness under vacuum. The crude product is suspended in 50 ml of hydrochloric acid 1N, extracted three times with 20 ml ethyle acetate; the organic phases are washed twice with 15 ml water, collected, dried on anhydrous sodium sulphate and evaporated under vacuum. The crude product is crystallized from 30 ml of cold hexane. The crystallized

25

- 15 -

product is separated by filtration, washed twice with 5 ml hexane and dried under high vacuum.

The reaction yield is about 87%.

The physico-chemical properties of N-lauroyl-L-serine methylester are

5 as follows:

-physical state: white crystalline powder  
-molecular formula:  $C_{16}H_{31}NO_4$   
-molecular weight: 301.43  
-elemental analysis: C= 63.75% H= 10.37% N= 4.65% O= 21.23%

10 -solubility in org. sol.: >10 mg/ml in DMSO  
>10 mg/ml in ethanol

-water solubility: slightly soluble

-melting point: 63-65°C

-TLC: eluent: toluene/ethanol/acetic acid

15 65:30:5 Rf= 0.60

#### EXAMPLE 6: Preparation of N-palmitoyl-L-cysteine

1.82g of L-cysteine (15 mmol) are solubilized at 4°C in 60 ml of  $K_2CO_3$  1M. The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.75 g of palmitoyl chloride (10 mmol). The  
20 resulting mixture is maintained under stirring at 0°C overnight. therefater is acidified with hydrochloric acid 6N. The crude precipitate is separated by filtration, dried under vacuum and crystallized a first time from 30 ml of ter-butyl-methyl-ether and thereafter from 30 ml of cold methanol. The crystallized product is  
25 separated by filtration, washed twice with 5 ml methanole and dried under high vacuum.

The reaction yield is about 92%.

- 16 -

The physico-chemical properties of N-palmitoyl-L-cysteine are as follows:

- physical state: white crystalline powder
- molecular formula:  $C_{19}H_{37}NO_3S$
- 5 -molecular weight: 359.57
- elemental analysis: C= 63.47%, H= 10.37%, N= 3.90%,  
O= 13.35%, S= 8.92%
- solubility in org. sol.: >10 mg/ml in DMSO
- water solubility: slightly soluble
- 10 -melting point: -
- TLC: eluent: chloroform/methanol/water/  
 $NH_3$  (28%) 80:25:2:1 Rf: 0.42

**EXAMPLE 7: Preparation of N-palmitoyl-glycine**

- 1.13 g of glycine (15 mmol) are solubilized at 4°C in 40 ml of KOH 1N.
- 15 The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.75 g of palmitoyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight, thereafter is acidified with hydrochloric acid 6N. The crude precipitate is separated by filtration, dried under vacuum and crystallized from 60
- 20 ml of cold ethanol 95°. The crystallized product is separated by filtration, washed twice with 5 ml ethanol 95° and dried under high vacuum.

The reaction yield is about 79%.

The physico-chemical properties of N-palmitoyl-glycine are as follows:

- 25 -physical state: white crystalline powder
- molecular formula:  $C_{18}H_{35}NO_3$
- molecular weight: 313.48

- 17 -

- elemental analysis: C= 68.97% H= 11.25% N= 4.47% O= 15.31%
- solubility in org. sol.: >10 mg/ml in DMSO
- water solubility: slightly soluble
- melting point: 121-123°C
- 5 -TLC: eluent: chloroform/methanol/water  
NH<sub>3</sub> (28%) 80:25:2:1 Rf: 0.22

**EXAMPLE 8: Preparation of N-lauroyl-glycine**

1.13 g of glycine (15 mmol) are solubilized at 4°C in 40 ml of KOH 1N. The solution is slowly added, dropwise under stirring over a period of  
10 30 minutes, with 2.19 g of lauroyl-chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight, thereafter is acidified with hydrochloridic acid 6N and extrated three times with 20 ml ethyle acetate; the organic phases are collected, dried with anhydrous sodium sulphate and evaporated under vacuum. The crude  
15 product is crystallized from 30 ml of cold acetonitrile. The crystallized product is separated by filtration, washed twice with 5 ml acetonitrile and dried under high vacuum.

The reaction yield is about 83%.

The physico-chemical properties of N-lauroyl-glycine are as follows:

- 20 -physical state: white crystalline powder
- molecular formula: C<sub>14</sub>H<sub>27</sub>NO<sub>3</sub>
- molecular weight: 257.37
- elemental analysis: C= 65.34% H= 10.57% N= 5.44% O= 18.65%
- solubility in org. sol.: >10 mg/ml in DMSO
- 25 >10 mg/ml in ethanol
- water solubility: slightly soluble (>10 mg/ml in phosphate buffer 50 mM, pH 7.4, NaCl 0.9%)

- 18 -

-melting point: 117-119°C  
-TLC: eluent: toluene/ethanol/acetic acid  
65:30:5 Rf = 0.56

**EXAMPLE 9: Preparation of N-lauroyl-glycine methylester**

5 1.88 g of glycine methyl ester hydrochloride (15 mmol) are solubilized at 4°C in 40 ml of a mixture tetrahydrofuran/water 9:1 with 4.05 g of triethylamine (40 mmol). The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.19 g of lauroyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C  
10 for 2 h and at room temperature for a night and evaporated to dryness under vacuum. The crude product is suspended in 50 ml of hydrochloric acid 1N, extracted three times with 20 ml ethyle acetate; the organic phases are washed twice with 15 ml water, collected, dried on anhydrous sodium sulphate and evaporated under vacuum. The crude is  
15 crystallized from 30 ml of cold hexane. The crystallized product is separated by filtration, washed twice with 5 ml hexane and dried under high vacuum.

The reaction yield is about 89%.

The physico-chemical properties of N-lauroyl-glycine methylester are  
20 as follows:

-physical state: white crystalline powder  
-molecular formula:  $C_{15}H_{29}NO_3$   
-molecular weight: 271.40  
-elemental analysis: C= 66.38% H= 10.77% N= 5.16% O= 17.69%  
25 -solubility in org. sol.: >10 mg/ml in DMSO  
>10 mg/ml in ethanol  
-water solubility: slightly soluble

- 19 -

-melting point: 62-64°C  
-TLC: eluent: toluene/ethanol/acetic acid  
65:30:5 Rf= 0.63

**EXAMPLE 10: Preparation of N-palmitoyl-β-alanine**

5 1.34g of β-alanine (15 mmol) are solubilized at 4°C in 40 ml of KOH 1N. The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.75 g of palmitoyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight, acidified with hydrochloric acid 6N and extracted three times with 20  
10 ml ethyle acetate; the organic phases are washed twice with 15 ml water, collected, dried on anhydrous sodium sulphate and evaporated under vacuum. The crude product is crystallized from 30 ml of ter-butyl-methyl-ether. The crystallized product is separated by filtration, washed twice with 10 ml ter-butyl-methyl-ether and dried  
15 under high vacuum.

The reaction yield is about 93%.

The physico-chemical properties of N-palmitoyl-β-alanine are as follows:

-physical state: white crystalline powder  
20 -molecular formula:  $C_{19}H_{37}NO_3$   
-molecular weight: 327.51  
-elemental analysis: C= 69.68% H= 11.39% N= 4.28% O= 14.66%  
-solubility in org. sol.: >5 mg/ml in DMSO; >5 mg/ml in ethanol  
-water solubility: slightly soluble  
25 -melting point: 120 - 122°C  
-TLC: eluent: toluene/ethanol/acetic acid 65:30:5 Rf: 0.64

- 20 -

**EXAMPLE 11: Preparation of N-palmitoyl-gamma-aminobutyric acid**

1.55 g of gamma-amino-butyric acid (15 mmol) are solubilized at 4°C in 40 ml of KOH 1N. The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.75 g of palmitoyl-  
5 chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight, acidified with hydrochloric acid 6N and extracted three times with 20 ml ethyle acetate; the organic phases are washed twice with 15 ml water, collected, dried with anhydrous sodium sulphate and evaporated under vacuum. The crude product is  
10 crystallized from 30 ml of ter-butyl-methyl-ether. The crystallized product is separated by filtration, washed twice with 10 ml ter-butyl-methyl-ether and dried under high vacuum.

The reaction yield is about 90%.

The physico-chemical properties of N-palmitoyl-gamma-aminobutyric acid  
15 are as follows:

- physical state: white crystalline powder
- molecular formula:  $C_{20}H_{39}NO_3$
- molecular weight: 341.54
- elemental analysis: C= 70.34% H= 11.51% N= 4.10% O= 14.05%
- 20 -solubility in org. sol.: >5 mg/ml in DMSO; >5 mg/ml in ethanol
- water solubility: slightly soluble
- melting point: 104 - 106°C
- TLC: eluent: toluene/ethanol/acetic acid  
65:30:5 Rf: 0.64

25 **EXAMPLE 12: Preparation of N,N'-bis-(1-carboxy-2-hydroxyethyl)-nonandiamide**

4.66 g of L-serine methylester hydrochloride (30 mmol) are solubilized

- 21 -

at 4°C in 80 isopropanole with 8.1 g of triethylamine (80 mmol). The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.25 g of azelaoyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C for 2 h and at room temperature for a night and evaporated to dryness under vacuum. The crude product is suspended in 30 ml of water, extracted three times with 20 ml ethyle acetate; the organic phases are washed twice with 15 ml water, collected, and evaporated under vacuum. The crude product is suspended in 50 ml of water, under continuous stirring, and added with 20 ml NaOH 1M to hydrolyze the esteric groups. After 30 minutes the solution is cooled to 4°C and acidified with hydrochloric acid 6N. The precipitated crude is separated by filtration, crystallized from 30 ml of a cold mixture acetonitrile/water 9:1. The crystallized product is separated by filtration, washed twice with 5 ml acetonitrile and dried under high vacuum.

The reaction yield is about 75%.

The physico-chemical properties of N-N'-bis-(1-carboxy-2-hydroxyethyl)-nonandiamide are as follows:

- physical state: white crystalline powder
- 20 -molecular formula:  $C_{15}H_{26}N_2O_8$
- molecular weight: 362.38
- elemental analysis: C= 49.72% H= 7.23% N= 7.73% O= 35.32
- solubility in org. sol.: >10 mg/ml in DMSO
- >10 mg/ml in ethanol
- 25 -water solubility: slightly soluble (>10 mg/ml as sodium salt)
- melting point: 89-93°C



- 22 -

-TLC: eluent:                    acetonitrile/water/acetic acid  
4:1:0.1    Rf= 0.31

EXAMPLE 13: Preparation of N,N'-trans-2-dodecendioyl-diglycine

2.28 g of traumatic acid (10 mmol) are solubilized in 40 ml of  
5 anhydrous dimethylformamide at 0°C with 2 ml of pyridine. 2.53 g of N-  
hydroxysuccinimide (22 mmol) and 4.12 g of dicyclohexylcarbodiimide  
(20 mmol) are added and the resulting mixture is stirred for 2 h at  
0°C. The formed urea is separated by filtration and discarded, while  
the solution containing the succinimidic ester is stirred again at 0°C  
10 and added with 2.25 g of glycine (30 mmol) and 3.04 g of triethylamine  
(30 mmol).

The resulting mixture is stirred for a night at room temperature and  
evaporated to dryness under vacuum. The residue is solubilized in 50  
ml of water and the solution is acidified with hydrochloric acid 6N.  
15 The crude precipitate is collected by filtration, dried under vacuum  
and crystallized from 60 ml of methanol. The crystallized product is  
separated by filtration, washed twice with 5 ml of methanol and dried  
under high vacuum.

The reaction yield is 80%.

20 The physico-chemical properties of N,N'-trans-2-dodecendioyl-diglycine  
are as follows:

-physical state:                    white crystalline powder  
-molecular formula:                 $C_{16}H_{26}N_2O_6$   
-molecular weight:                 342.39  
25 -elemental analysis:             C= 56.13% H= 7.65% N= 8.18% O= 28.04  
-solubility in org. sol.: >10 mg/ml in DMSO  
-water solubility:                 slightly soluble (>10 mg/ml as sodium

- 23 -

salt)

-melting point: 194°C (dec)

-TLC: eluent: acetonitrile/water/formic acid  
4:1:0.1 Rf= 0.85

5 **EXAMPLE 14: Preparation of N-palmitoyl-D-glucosamine**

3 23 g of D-glucosamine hydrochloride (15 mmol) are solubilized at 4°C  
in 60 ml of K<sub>2</sub>CO<sub>3</sub> 1M. The solution is slowly added, dropwise under  
stirring over a period of 30 minutes, with 2.75 g of palmitoyl  
chloride (10 mmol). The resulting mixture is maintained under stirring  
10 at 0°C overnight and acidified to neutrality with hydrochloric acid  
6N. The precipitated crude product is separated by filtration and  
dried under vacuum; thereafter it is purified with repeated washing  
with warm ethanol, recovered by filtration and dried under high  
vacuum.

15 The reaction yield is about 78%.

The physico-chemical properties of N-palmitoyl-D-glucosamine are as  
follows:

-physical state: white amorphous powder

-molecular formula: C<sub>22</sub>H<sub>43</sub>NO<sub>6</sub>

20 -molecular weight: 417.58

-elemental analysis: C= 63.28% H= 10.38% N= 3.35% O= 22.99%

-solubility in org. sol.: >1 mg/ml in DMSO

-water solubility: slightly soluble

-melting point: 202°C (dec)

25 -TLC: eluent: chloroform/methanol/water  
NH<sub>3</sub> (28%) 80:25:2:1 Rf: 0.30

**EXAMPLE 15: Preparation of N-lauroyl-D-glucosamine**

- 24 -

3.23g of D-glucosamine hydrochloride (15 mmol) are solubilized at 4°C in 60 ml of K<sub>2</sub>CO<sub>3</sub> 1M. The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.19 g of lauroyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight and acidified to neutrality with hydrochloric acid 6N. The precipitated crude product is separated by filtration and dried under vacuum; thereafter it is purified with repeated washing with warm isopropanol, recovered by filtration and dried under high vacuum.

The reaction yield is about 85%.

- 10 The physico-chemical properties of N-lauroyl-D-glucosamine are as follows:

-physical state: white amorphous powder

-molecular formula: C<sub>18</sub>H<sub>35</sub>NO<sub>6</sub>

-molecular weight: 361.48

- 15 -elemental analysis: C= 59.81% H= 9.76% N= 3.87% O= 26.56%

-solubility in org. sol.: >5 mg/ml in DMSO

-water solubility: slightly soluble

-melting point: >170°C (dec)

-TLC: eluent: toluene/ethanol/acetic acid

- 20 65:30:5 Rf: 0.31

#### EXAMPLE 16: Preparation of N-oleoyl-D-glucosamine

3.23 g of D-glucosamine hydrochloride (15 mmol) are solubilized at 4°C in 30 ml of K<sub>2</sub>CO<sub>3</sub> 1M. The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 3.01 g of oleoyl chloride (10 mmol). The resulting mixture is maintained under stirring at 0°C overnight, acidified with hydrochloric acid 6M and extracted in continuous with ethyle acetate. The extraction solution is evaporated

- 25 -

to dryness and the crude product is purified by silica-gel chromatography, using as eluent a mixture of chloroform/methanol/water/ $\text{NH}_3$  (25%) 85:15:1:0.5; the fractions containing the pure product are collected, evaporated to dryness and  
5 the residue is dried under high vacuum.

The reaction yield is about 86%.

The physico-chemical properties of N-oleoyl-D-glucoseamine are as follows:

- physical state: white amorphous powder
- 10 -molecular formula:  $\text{C}_{24}\text{H}_{45}\text{NO}_6$
- molecular weight: 443.62
- elemental analysis: C= 64.98% H= 10.22% N= 3.16% O= 21.64%
- solubility in org. sol.: >5 mg/ml in DMSO
- water solubility: slightly soluble
- 15 -melting point: -
- TLC: eluent: chloroform/methanol/water  
 $\text{NH}_3$  (25%) 80:25:2:1 Rf: 0.35

**EXAMPLE 17: Preparation of N-lauroyl-4-hydroxy-L-proline**

1.97 g of 4-hydroxy-L-proline (15 mmol) are solubilized at 4°C in 50  
20 ml of water containing 3.18 g of  $\text{Na}_2\text{CO}_3$  (30 mmol). The solution is slowly added, dropwise under stirring over a period of 30 minutes, with 2.19 g of lauroyl-chloride (10 mmol) in 50 ml of petroleum ether. The resulting mixture is kept under stirring for 4 hours at 0°C and overnight at room temperature. The resulting mixture is acidified with  
25 HCl 6N and extrated three times with 50 ml ethyle acetate; the organic phases are washed 2 times with 15 ml of water, collected, dried with anhydrous sodium sulphate and evaporated under vacuum. The crude

- 26 -

product is crystallized from 50 ml of cycloesane. The crystallized product is separated by filtration, washed twice with 10 ml cycloesane and dried under vacuum.

The reaction yield is about 85%.

- 5 The physico-chemical properties of N-lauroyl-4-hydroxy-L-proline are as follows:

- physical state: white crystalline powder
- molecular formula:  $C_{17}H_{31}NO_4$
- molecular weight: 313.44
- 10 -elemental analysis: C= 65.14% H= 9.97% N= 4.47% O= 20.42%
- solubility in org. sol.: >10 mg/ml in DMSO  
>10 mg/ml in ethanol
- water solubility: slightly soluble (>10 mg/ml in phosphate buffer 100 mM, pH 7.4)
- 15 -melting point: 86.5-88.5°C
- TLC: eluent: chloroform/methanol/water/ $NH_3$  (28%)  
80:25:2:1 Rf = 0.22

**EXAMPLE 18: Preparation of N-palmitoyl-D-glucosamine tetra-O-emisuccinate**

- 20 4.18 g of N-palmitoyl-D-glucosamine (10 mmol) are solubilized at 4°C in 40 ml of anhydrous pyridine; the resulting solution is added with 4.4 g of succinic anhydride (40 mmol) and kept under stirring at 0°C over 2 hours and overnight at 40°C. The solution is evaporated to dryness under vacuum, the residue is taken up with 20 ml HCl 1N and
- 25 extracted 3 times with 50 ml of ethyle acetate; the organic phases are washed 2 times with 15 ml of water, collected, dried with anhydrous sodium sulphate and concentrated under vacuum to a volume of about 50

- 27 -

ml. The resulting solution is added with petroleum ether to muddiness and cooled at 4°C. The obtained crystallized product is separated by filtration, washed 2 times with 10 ml of petroleum ether and dried in high vacuum.

5 The reaction yield is about 93%.

The physico-chemical properties of N-palmitoyl-D-glucosamine tetra-O-emisuccinate are as follows:

- physical state: white crystalline powder
- molecular formula:  $C_{38}H_{59}NO_{18}$
- 10 -molecular weight: 817.88
- elemental analysis: C= 55.81% H= 7.27% N= 1.71% O= 35.21%
- solubility in org. sol.: >10 mg/ml in DMSO  
>10 mg/ml in ethanol
- water solubility: slightly soluble (>10 mg/ml in phosphate  
15 buffer 100 mM, pH 7.4)
- melting point: 131°C (dec.)
- TLC: eluent: toluene/ethanol/acetic acid  
80:20:2 Rf = 0.27

As already mentioned, the amides of mono and bicarboxylic acids with  
20 aminoalcohols and aminoethers according to the present invention, corresponding to formula (I) reported hereinabove, can selectively bind to CB2 peripheral receptor, acting as competitive agonists and displacing from said receptor the known natural and synthetic cannabinoids already known. Furthermore, said amides are capable of  
25 functionally activating CB2 receptor, by mimicking the non-psychoactive biological effects of cannabinoids and acting with a potency higher than or comparable with the one of cannabinoids.

- 28 -

The Applicant has also surprisingly found that CB2 cannabinoid peripheral receptor is present on mast cell and therefore the new compounds according to the present invention act on the same mast cell functionality. Furthermore, the Applicant has unexpectedly found that  
5 CB2 cannabinoid peripheral receptor is functionally expressed also on non-immune cells or tissue; in particular the Applicant has found that CB2 is functionally expressed in the Nervous System on cerebellar granule cells, indicating that CB2 receptor can modulate also non-immune cell function.

10 This extremely important aspect of the present invention will be illustrated in detail hereinbelow.

A) Identification of CB2 receptor on mast cells and non-immune cells.

In order to verify that CB2 cannabinoid peripheral receptor is present on mast cells and non-immune cells (i.e. neurons), the presence of  
15 messenger RNA for said receptor was investigated in primary cultures of rat peritoneal mast cells, in a basophilic-mastocyte cell line from rat solid tumour (Rat Basophilic Leukemia, RBL-2H3) and in mouse cerebellar granule cell culture, in comparison with rat spleen homogenate tissues, where the presence of said receptor is  
20 acknowledged.

Both Polymerase Chain Reaction and in situ hybridisation technique were used.

i) Cannabinoid Peripheral Receptor specific hybridisation amplified by Polymerase Chain Reaction (PCR).

25 Extraction of total mRNA from tissues and cells

Total RNA was extracted from rat peritoneal mast cells, RBL-2H3 cells and rat spleen, according to the method described by Chumczynski and

- 29 -

Sacchi (Anal. Biochem., 162, 156-159, 1987).

5 · 10<sup>6</sup> mast cells, cells RBL-2H3 and 50 mg of spleen were separately homogenized in a 1.5 ml polypropylene tube Eppendorf® (safe-lock type) with guanidinium isothiocyanate 5M (600 µl). Each tube was then supplied with sodium acetate 2M (60 µl), pH 5, and phenol (600 µl) saturated in buffer Tris- HCl 1M, pH 7.0. The samples were briefly stirred by vortex mixing (Heidolph, type REAX 2000®) and then added with chloroform-isoamyl alcohol (49:1 v/v) (120 µl). After agitation for 15 sec. by vortex mixing and incubation in ice for 15 min, the samples were centrifuged at 15,000xg at 4°C for 15 min. 600 µl of the upper aqueous phase was withdrawn and placed in another tube, having care to avoid any contact with the interface of the organic lower phase, which was discarded. The aqueous phase was added with isopropanol (600 µl) and, after agitation, the samples were incubated at -20°C for at least 60 min and centrifuged at 15,000xg at 4°C for 5 min. The supernatant was discarded. The pellet was washed with ethanol at 80% and then with absolute ethanol, concentrated to dryness and suspended again in water (20 µl). 2 µl of the sample were used for the spectrophotometric determination of RNA concentration at the wavelength of 260 nm and for the visualization with ethidium bromide on agarose gel at 1%.

Reverse transcription and amplification by Polymerase Chain Reaction (PCR) of CB2 receptor

1 µg of total RNA withdrawn from each of the above samples was subjected to reverse transcription, as described by Leon et al. (PNAS, 91:3739-3743, 1994), using the reverse transcriptase of murine moloney leukaemic virus and 50 pmol of the synthetic oligonucleotide SEQ ID



- 30 -

NO:1. having sequence 5'-TAGGTAGGAGATCAAGCG-3', complementary and antiparallel to the mRNA coding for cannabinoid peripheral receptor (Munro et al., Nature 365, 61- 65, 1993), in a total volume of 20 µl.

After 1-hr reaction at 37°C, the volume of the transcription product

5 was brought to 100 µl with water and one fourth of the final volume was subjected to amplification by means of PCR. Said operation was conducted in 500 µl thin-walled test tubes, filled up to a final volume of 100 µl with a solution having the following composition:

50 mM TRIS HCl, pH 8.3, at 25°C:

10 75 mM KCl;

2.5 mM MgCl<sub>2</sub>;

10 mM DTT (dithiothreitol);

0.2 mM dATP, dCTP, dGTP and dTTP;

50 pmol sense primer SEQ ID NO:2 (5'-TTTCACGGTGTGGACTCC-3');

15 50 pmol antisense primer SEQ ID NO:3 (5'-TAGGTAGGAGATCAAGCG-3');

0.551 Taq Pol (Taq polymerase) 2.5 U/µl (Polymerase Stoffel fragment, Cetus/Perkin-Elmer®);

3% formamide.

Primers were specific for cannabinoid peripheral receptor. PCR was

20 carried out for 35 thermal cycles in apparatus mod. 9600 of Cetus/Perkin Elmer®, according to the following procedure:

Temperature	Duration
-------------	----------

95°C	1 min
------	-------

54°C	1 min
------	-------

25 72°C 1 min

Once the reaction had been completed, 30 µl of the reaction volume was electrophoresed on 1% agarose gel in order to visualize the

- 31 -

amplification products, then transferred and immobilized on a nylon filter for inner-probe hybridization to the two PCR primers.

Specific hybridization with inner oligonucleotide

The identity of the amplification product was determined by  
5 hybridization with a synthetic oligonucleotide SEQ ID NO:4, complementary to amplified sequences of cannabinoid peripheral receptor, labelled with radioactive tracers.

The sequence SEQ ID NO:4 was as follows:

5'-GGTGACGAGAGCTTTGTAGGTAGGTGGGTAGCACAGACATAGGTA-3'

10 Radioactive labelling was carried out at 37°C for 1 hr, using 5 pmol inner oligonucleotide, as follows: 33 pmol ( $\alpha^{32}\text{P}$ )-dATP (New England Nuclear);

20 U Terminal Deoxynucleotide Transferase (US Biochemical);

100 mM sodium cacodylate (pH 7.2)

15 2 mM  $\text{CoCl}_2$

0.2 mM 2-mercaptoethanol

in a final volume of 20  $\mu\text{l}$ .

The labelled products were purified by chromatography on Sephadex G-50<sup>®</sup>

column, and added to the hybridization solution containing the  
20 filter with the PCR-amplified products. Hybridization and washings were carried out under standard conditions, as described by Sambrook, Fritsch and Maniatis (Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989).

The hybridized filter was exposed to a autoradiographic film for the  
25 detection of the amplified bands of the cannabinoid peripheral receptor messenger.

- 32 -

ii) m-RNA - Peripheral Cannabinoid Receptor in situ hybridisation.

The brains of adult male mice (20-22 g) were frozen in 2-methyl-butane at -80°C, sectioned with a cryostat and the 12 mm coronal sections were thaw-mounted on poly-L-lysine coated slides. All sections were  
5 then fixed in 4% paraformaldehyde (PFA) and dehydrated in a graded series of ethanol, including a 5 min. incubation in chloroform and air dried. Mouse cerebellar granule cells (15 day in vitro-DIV) cultured on poly-L-lysine coated coverslips were fixed in 4% PFA, washed twice in PBS and permeabilized in 70% ethanol at 4°C for 48 hrs, dehydrated  
10 in higher graded ethanol and air dried. To detect the CB2-specific mRNA the synthetic oligonucleotide (45 mers) CB2 (SEQ ID NO:4: 5'-GGTGACGAGAGCTTTGTAGGTAGGTGGGTAGCACAGACATAGGTA-3 according to Munro et al., 1993, cited reference) was chosen.

A random sequence was utilized for control sections. All  
15 oligonucleotides were tailed with <sup>35</sup>S-dATP (NEN) using terminal deoxynucleotidyl-transferase (Pharmacia) to a specific activity of 10<sup>9</sup> cpm/mg. All sections were hybridized in standard solutions (\*) with 1.5 10<sup>7</sup> dpm/ml overnight at 42°C in a humidified chamber. Sections were then washed once in 1X SSC/0.1%(\*\*) SDS at 55°C for 30 min., then  
20 twice in 1X SSC at 55°C for 15 min., followed by 0.1X SSC at 25°C for 30 min., rinsed in autoclaved water for 2min., dehydrated in ethanol and air dried. The slides were subsequently dipped in photoemulsion (Ilford K.5 diluted 1:1 in water), exposed for 5 weeks at 4°C, developed (Ilford Phenisol), fixed (Ilford Hypam) and counterstained  
25 with cresyl violet.

Standard Solution preparation

(\*) Hybridisation cocktail Formamide: 5 ml. 50%; SSC 20x: 2 ml. 4 x;

- 33 -

Denhardt's 50x: 0.2 ml; 1x Lauryl-saccharosyl 20%: 0.5 ml, 1%; Dextran sulphate: 1 gr, 10% ; 200 mM Phosphate buffer pH 7.0: 1 ml, 20 mM, water-DEPC q.s. to 10 ml. Filtrate using 0.45  $\mu$ m filter and store at -20°C .

- 5 (\*\*) Sodium Citrate Solution SCS 20x. Sodium chloride 175.3g and sodium citrate 88.2g were dissolved in 800ml DEPC-water; NaOH 10M was used to adjust the pH 7.2. Add DEPC-water q.s. to 1000ml for autoclave procedure.

The autoradiographic examination shows that rat peritoneal mast cells  
10 and the cousin RBL-2H3 cells, cerebellar granule cells and cerebellum express, similarly to spleen preparations, mRNA specific to peripheral cannabinoid receptor CB2. This finding is clearly shown in Figures 1 and 2. Figure 1 illustrates the specific hybridization of cannabinoid peripheral receptor amplified by Polymerase Chain Reaction (PCR)  
15 respectively in (A) rat spleen. (B) rat peritoneal mast cell cultures and (C) RBL-2H3 cell cultures (C). Figure 2 illustrates the specific mRNA-CB2 peripheral cannabinoid receptor in situ hybridisation.

#### B) Binding assays

Specific binding assays were conducted for the purpose of checking  
20 whether the receptor was functionally expressed and whether the amides forming the object of the present invention were able to bind to CB2 receptor in a specific and competitive manner, compared with the cannabinoids having a known affinity to the receptor and compared with anandamide, the endogenous ligand of CB1 receptor.

- 25 a) Preparation of plasmatic membranes of RBL-2H3 cells

RBL-2H3 cells ( $100 \times 10^6$ ), frozen at -80°C, were thawed with 4 ml of Tris-HCl buffer solution 50 mM (pH 7.4), added with 0.25% w/v of

- 34 -

Trypsin inhibitor, type II-S: soy bean <sup>®</sup> (distributed by Sigma). The cells were resuspended in the buffer and homogenized. The homogenate was centrifuged at 1500xg at 4°C for 10 min. The supernatant obtained was collected and the precipitate was resuspended in 4 ml of the  
5 previous buffer. The resuspended precipitate was homogenized and centrifuged again at 1500xg at 4°C for 5-10 min. The resulting supernatant was combined to the supernatant previously obtained and centrifuged at 5000xg for 10 min. After centrifugation, the supernatant was collected and further centrifuged at 40,000xg at 4°C  
10 for 30 min. The resulting precipitate was resuspended in 0.5 ml of the buffer solution described above, with addition of 1% Bovine Serum Albumin fatty acid free. The obtained suspension was centrifuged at 40,000xg at 4°C for 30 min. The resulting precipitate was collected and resuspended in buffer solution containing 50 mM Tris HCl, 1 mM  
15 Tris-EDTA, 3 mM MgCl<sub>2</sub>, pH 7.4, in order to obtain a proteic concentration of 1µg/µl approx. This preparation may be used fresh, or frozen at -80°C and utilized within few days.

b) "Binding" assays conditions to the preparation of RBL-2H3 cell membranes

20 Saturation curve of labelled receptor agonist 3H-WIN 55,212-2 (specific activity 44 Ci/mmol, distributed by New England Nuclear): silanized plastic test tubes were fed, in the order, with binding buffer (50 mM Tris HCl, 1 mM Tris-EDTA, 3 mM MgCl<sub>2</sub>, 0.5% w/v Bovine Serum Albumin fatty acid-free) to a final volume of 0.5 ml, decreasing  
25 doses of 3H-WIN 55,212-2 from 0.5 to 20 nM (final) and 30 µg membrane proteins prepared in item a). The resulting binding mixture was incubated at 30°C for 60 min under stirring and centrifuged at

- 35 -

40.000xg at 20°C for 15 min. After centrifugation, an aliquot portion of the supernatant was collected to calculate the concentration of the ligand not associated to the membranes. After removal of the supernatant residue, the precipitate was washed with 1 ml PBS  
5 (Phosphate Buffer Solution) containing 0.5% Bovine Serum Albumin, taken up with 50 µl of a mixture of ethanol and 1% Triton X-100 (R) (50/50 v/v), incubated at 37°C for 20 min and resuspended.

The resuspended material was added and mixed with scintillator liquid (3 ml) and placed in a "β-counter" for 5 min. To evaluate the  
10 "Aspecific Binding", the predetermined test tubes were fed first with unlabelled receptor agonist WIN 55,212-2 at a final concentration of 15 nM and then with the labelled ligand. Said binding gives  $K_d = 5-10$  nM and  $B_{max} = 100-250$  pM.

3H-WIN 55,212-2 binding displacement

15 For these competitions, 3H-WIN 55,212-2 was used at a concentration of 3 µM; non-radioactive WIN 55,212-2 (1µM) was used to inhibit specific binding. The competitors Nabilone [3-(1,1-dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-1-hydroxy-6,6-dimethyl-9H-dibenzo-[b,d]-pyran-9-one] and anandamide were solubilized in 0.1% ethanol, whereas  
20 the products forming the object of the present invention were solubilized in 0.1% DMSO. The competitors were added to the binding mixture prior to the labelled ligand. In both cases, for an evaluation of the reference specific binding, ethanol and DMSO were added at the same final concentration to the total binding and to the aspecific  
25 one, also in the absence of competitors.

The aforementioned binding assays provided evidence that the receptor expression was complete and functional.

- 36 -

The data of Table 1 show that the amidic derivatives according to the present invention, like anandamide and the cannabinoid Nabilone, are able to bind to the receptor and to compete, with different potency, with the synthetic radioligand WIN 55,212-2.

Table 1 - Competitive inhibition of [ $^3\text{H}$ ] WIN 55,212-2 specific binding by the compounds of the invention. [ $^3\text{H}$ ] WIN 55,212-2 was used at a concentration of 3  $\mu\text{M}$ , whereas the other compounds were used at a concentration of 10  $\mu\text{M}$ .

Competitor	Specific binding	Displacement (%)
0.1% DMSO*	1433	—
Example 1	—	100
Example 7	297	79
0.1% Ethanol**	1843	—
Anandamide	99	95
Nabilone	—	100

\* 0.1% DMSO is the concentration used for amides solubilization according to the present invention.

\*\* 0.1% ethanol is the concentration used for Anandamide and Nabilone solubilization.

- 37 -

Table 2 - Competitive inhibition of [ $^3\text{H}$ ] WIN 55,212-2 binding to RBL-2H3 cell membranes by the amides of the invention and by the cannabinoids. Incubation was carried out in the presence of 1% DMSO. Inhibitory Concentration 50 values ( $\text{IC}_{50}$ ) are expressed as means  $\pm$  SEM, after the number of experiments indicated.

Compound	$\text{IC}_{50}$ (nM)	no. of experiments
Nabilone	$2.6 \pm 1.4$	4
$\Delta$ 8-THC	$223 \pm 120$	6
Cannabidiol	$> 1000$	3
Anandamide	$33 \pm 29$	5
Example 1	$0.04; 0.05$	2
Example 7	$0.16; 0.005$	2
Example 14	$8.9 \pm 4.8$	4

### C) Biological activity assays

The biological activity deriving from CB2 receptor functional activation was evaluated in RBL-2H3 cultures and in mouse cerebellar granule cell primary cultures. In particular, the ability of the amides of the invention to inhibit serotonin release, induced by specific stimulus, was evaluated on RBL cultures and compared with cannabinoids of known potency and with anandamide.

The specific binding and/or the functional effects of the amidic derivatives described in Examples 1, 2, 5-11 and 13-18 were evaluated.

For purpose of comparison, the following products were used: among natural cannabinoids, three different isomers of tetrahydrocannabinol (THC):  $\Delta$  8-THC,  $\Delta$  9-THC and 11-nor- $\Delta$  8-THC-9; among synthetic cannabinoids, Nabilone and WIN 55,212-2, in addition to the CB1



- 38 -

endogenous ligand anandamide. Furthermore, it was shown that CB2 receptor plays a role in protecting against Excitatory Amino Acid (EAA)-induced cell death.

#### Preparation of RBL-2H3 cell cultures

- 5 RBL-2H3 cell cultures were grown in flasks (Falcon®), at 37°C, 5% CO<sub>2</sub>, in the presence of Minimum Essential Eagle's Medium (MEM) and 4 mM glutamine, 100 U/ml penicillin and 20% deactivated fetal calf serum (DFCS).

#### RBL-2H3 cell sensitization and activation

- 10 For each test, the cells were removed from the flasks with PBS, containing 0.5 mM EDTA at pH 7.2, and seeded in wells (96 well plate Falcon®) in the presence of RPMI-1640 medium (code 6504, distributed by Sigma) containing 50 mg/l gentamicin and 10% FCS at a density of 100,000 cells/100 µl medium/well. Furthermore, during seeding, said
- 15 cells were loaded with serotonin by addition of 1 µCi/ml 3H-serotonin (5-hydroxy-triptamine 5HT, 26.4 Ci/mmol, New England Nuclear) and then incubated for 18 hrs (37°C, 5% CO<sub>2</sub>). After 18-hr incubation in the presence of 3H-serotonin, the culture medium was removed and the cells were sensitized for 1 hr (37°C, 5% CO<sub>2</sub>) in the presence of PIPES
- 20 (N,N'-bis-(2-ethanesulphonyl)-piperazine, code 3768, Sigma) -buffered saline (100 µl/well), pH 7.1, containing 0.3 µg/ml mouse monoclonal antibody (IgE) against DNP (ADNP).

After removal of the sensitization medium, cells were activated - or not activated (control) - by addition, at 37°C for 155 min, of PIPES

25 (100 µl/well), pH 7.1, containing - or not containing (control) - 0.1 µg/ml human albumin combined with dinitrophenyl (DNP-HSA) and containing a natural or synthetic cannabinoid or an amidic derivative

- 39 -

according to the present invention. After incubation, the medium was collected and centrifuged to determine the  $^3\text{H}$ -serotonin quantity released from DNP-HSA-stimulated or not stimulated cells. In parallel, adhered cells were solubilized with 1% Triton X-100 <sup>®</sup> in PBS (100  
5  $\mu\text{l}$ /well) in order to determine the quantity of  $^3\text{H}$ -serotonin present in the cells. In both cases, the quantity of  $^3\text{H}$ -serotonin was measured by liquid scintillography and radioactivity counting, using a  $\beta$ -counter of Canberra Packard, 1900 TR <sup>®</sup>.

Addition of cannabinoids or of the derivatives according to the  
10 present invention during RBL-2H3 cells activation

It was evaluated the effect of the amidic derivatives of the invention (stock solution in DMSO) and of cannabinoids in the presence or in the absence of anandamide (stock solution in ethanol) on DNP-HSA-induced  $^3\text{H}$ -serotonin release in DNP sensitized cells. To this purpose,  
15 cannabinoids or the amidic derivatives of the invention, with or without addition of anandamide, were added to the cell activation medium (PIPES  $\pm$  DNP-HSA) at the desired concentration. In all cases, the medium was incubated at 37°C for 15 min, while the total final concentration was kept constant (0.2% solvents).

20 The amides of the invention were solubilized in 0.2% DMSO and 0.1% absolute ethanol, except for N-palmitoyl-L-serine, which was solubilized in 1% DMSO and 0.1% ethanol.

Quantification of  $^3\text{H}$ -serotonin net release after RBL-2H3 cells  
activation

25  $^3\text{H}$ -serotonin release in the various samples was calculated according to the following formula:

- 40 -

$$\text{release} = \frac{\text{dpm released}}{(\text{dpm released} + \text{dpm associated with the cells})} \times 100$$

where "dpm" means nuclear disintegrations per minute.

The effect of the amides of the invention was expressed as the percentage of 3H-serotonin net release (i.e. after deduction of the percentage of release in the absence of DNP-HSA) or as the percentage of 3H-serotonin release inhibition.

The results of evaluation tests on the functional effects of cannabinoids and of the amidic derivatives of the invention are shown in Table 3. These data indicate that natural cannabinoids  $\Delta^8$ -tetrahydrocannabinol ( $\Delta^8$ -THC) and  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), the synthetic cannabinoid Nabilone and the labelled receptor agonist WIN 55,212-2 are able to inhibit, in a concentration-dependent way, RBL cells immunogenic activation measured as serotonin release. It is evident that the compounds described in Examples 1 and 7 can inhibit the effects induced by mast cell activation and exert an effect that is higher than or comparable to the one of cannabinoids.

Conversely, anandamide is unable to affect serotonin release, thus demonstrating a specific structural selectivity in the receptor functional activation.

The compounds derived from saturated carboxylic acids are more potent in exerting the functional effects following receptor activation and this is confirmed by the comparative tests results, reported below, wherein it is reported the effect of N-(2-hydroxyethyl)-hexadecanamide, wherein the acyl-moiety is sature, and that of N-(2-hydroxyethyl)-lnoleylamide, wherein the acyl-moiety presents two double bonds.

- 41 -

Table 3 - Effect of natural and synthetic cannabinoids and of the amides according to the present invention on serotonin release induced by ADNP/DNP.HSA immunogenic stimulation in RBL-2H3 cells. The tested compounds were solubilized in 0.1% DMSO and 0.1% ethanol, a part from N-(2-hydroxyethyl)-hexadecanamide which was solubilized in 1% DMSO and 1% ethanol.

Molecules added after sensitization with ADNP	<sup>3</sup> H-serotonin net release (%)	ED50 (μm)
DNP.HSA-	100	
DNP.HSA+WIN 55,212-2 (50μM)	0	6.4
DNP.HSA+Nabilone (10μM)	0	2.8
DNP.HSA+Δ <sup>8</sup> -THC (25μM)	0	5
DNP.HSA+Δ <sup>9</sup> -THC (25μM)	27	5
DNP.HSA+11-nor-Δ <sup>8</sup> -THC-9 (20μM)	33	15
DNP.HSA+anandamide	100	—
DNP.HSA+N-(2-hydroxyethyl)-hexadecanamide (100μM)	0	2.6
DNP.HSA+N-(2-hydroxyethyl)-linoleylamide (100μM)	100	—
DNP.HSA+Example 1 (100μM)	0	—
DNP.HSA+Example 7 (10μM)	27	4.8

Co-incubation of anandamide with natural or synthetic cannabinoids and with the derivative described in Example 7 reduces the ability of the acyl amide of the invention, as well as of cannabinoids, to inhibit serotonin release, as reported in Table 4.

- 42 -

**Table 4** - Anandamide antagonism towards the inhibitory effect of cannabinoids and amidic derivatives of the invention on ADNP/DNP.HSA-induced activation in RBL-2H3 cells.

Molecules added after sensitization with ADNP	3H-serotonin net release inhibition (%)	
	-anandamide	+ anandamide (12.5 $\mu$ M)
DNP.HSA+WIN 55,212-2 (30 $\mu$ M)	100	36
DNP.HSA+Nabilone (5 $\mu$ M)	90	50
DNP.HSA+ $\Delta$ 8-THC (25 $\mu$ M)	100	n.d.
DNP.HSA+ $\Delta$ 9-THC (25 $\mu$ M)	73	n.d.
DNP.HSA+11-nor- $\Delta$ 8-THC-9 (20 $\mu$ M)	67	n.d.
DNP.HSA+Example 7 (10 $\mu$ M)	73	24

The data reported in Table 5 show that the co-incubation of anandamide with natural or synthetic cannabinoids and with the amidic derivatives according to the present invention reduces the potency of anandamide, indicating that anandamide causes a competitive partial antagonism on the peripheral receptor. Therefore, as shown in Table 1, anandamide, though having binding affinity for the peripheral receptor, is not able to induce biological effects and, by competing for the receptor, functionally antagonizes the protective effects of cannabinoids.

- 43 -

Table 5 - ED<sub>50</sub> of cannabinoids and of the amidic derivatives of the invention on the inhibition of 3H-serotonin release induced by ADNP/DNP.HSA stimulation in RBL-2H3 cells, in the presence and in the absence of anandamide.

Molecules	ED50 (μM)	
	-anandamide	+anandamide (12.5 μM)
WIN 55,212-2	6.4	40
Nabilone	2.8	5
Δ <sup>8</sup> -THC	5	n.d.
Δ <sup>9</sup> -THC	5	n.d.
11-nor-Δ <sup>8</sup> -THC-9	15	n.d.
Example 7	4.8	>100
Example 14	0.51	3.30
Example 16	2.90	4.20

The partial competitive antagonism of anandamide on the peripheral receptor is exerted by the integral molecule and not by anandamide metabolites, as shown in Table 6.

- 44 -

Table 6 - <sup>3</sup>H-serotonin release inhibition by WIN 55,212-2 (used at a concentration of 30  $\mu$ m): antagonistic effect of anandamide vs. the metabolites of the same.

	3H-serotonin net release inhibition (%)	
	-WIN	+WIN
DNP.HSA	0	69
DNP.HSA + 12.5 $\mu$ M anandamide	7	15
DNP.HSA + 12.5 $\mu$ M ethanolamine	6	72
DNP.HSA + 12.5 $\mu$ M arachidonic acid	4	59
DNP.HSA + 12.5 $\mu$ M ethanolamide		
+ 12.5 $\mu$ M arachidonic acid	3	61

The tests results reported in Tables 4, 7 and 8 show that the receptorial specificity and the level of activity are functional to the nature of both the acylic and the amidic moieties of the amidic derivatives according to the present invention.

- 45 -

Table 7 - Inhibitory effect of the amidic derivatives according to the present invention on <sup>3</sup>H-serotonin release induced by ADP/DPN.HSA immunogenic stimulation in RBL-2H3 cells.

Compounds	3H-serotonin release inhibition %	
	DMSO 0.2%	DMSO 1%
Example 2	79 (100µM)	61 (100µM)
Example 5		61 (100µM)
Example 6	50 ( 60µM)	91 (100µM)
Example 7	85 ( 60µM)	100 (100µM)
Example 8	93 (100µM)	100 (100µM)
Example 9	55 (100µM)	
Example 10	47 (100µM)	
Example 13		54 (100µM)
Example 14	76 ( 20µM)	91 (100µM)
Example 15	67 ( 20µM)	86 (100µM)
Example 16	92 ( 20µM)	86 (100µM)

Numbers in parentheses are the concentrations (µM) used for the various compounds in the different tests.



- 46 -

**Table 8** - Inhibition of [ $^3\text{H}$ ] serotonin release from DNP-HSA activated RBL-2H3 cells. The compounds of the invention were present during the 15 minutes release period. EC50 is the concentration inhibiting by 50% the net release of [ $^3\text{H}$ ] serotonin from DNP-HSA - activated RBL-2H3 cells. The reported values are expressed as means  $\pm$  SEM, after the number of experiments shown in parentheses.

Compound	0.2% DMSO	EC50	1% DMSO
Example 5	104 $\pm$ 59 (2)		
Example 6			29 $\pm$ 12 (3)
Example 8	10 $\pm$ 1.5 (3)		7 $\pm$ 5 (2)
Example 10			30 (1)
Example 11	26 $\pm$ 18 (4)		
Example 14	1.28 $\pm$ 0.38 (6)		0.12 $\pm$ 0.07 (3)
Example 15	12 (1)		
Example 16	2.4 $\pm$ 0.5 (4)		

#### Cannabinoid neuroprotective effect in cerebellar granule cell culture

Granular cell cultures were obtained from postnatal day 8-9 mouse Balb-6 cerebellum. The cells were suspended in EBM + 2mM L-glutamine, 100 U/ml penicillin, 50 $\mu\text{g}/\text{ml}$  gentamycin, 25mM KCl and 10% fetal calf serum, plated on polylysine substrate, cultured from 8-10 days and then exposed to glutamate (500  $\mu\text{M}$ ) for 5 minutes at room temperature, exiting in 60% cell death. Cannabinoids and anandamide were solubilized in Locke's solution and added to the culture for 10 minutes, starting 15 minutes after removing glutamate. The number of

- 47 -

survived cells was calculated by the colorimetric method MTT, 24 hours after the washing out of the compound.

Cannabinoids protect against cell death induced by glutamate. To the contrary, anandamide, which can not functionally activate CB2  
5 cannabinoid peripheral receptor, does not display any protective effect, as shown in Table 9; anandamide was not effective up to 100  $\mu$ M. Furthermore, the data reported in Table 9 show that CB2 receptor is functionally expressed also on neurons culture, being able to play a protective role on cell survival; this indicates that CB2 receptor  
10 capability of modulating events is non-directly mediated by immune lineage cell.

Table 9 - Cannabinoid neuroprotective effect in comparison with anandamide. The reported values are expressed as means  $\pm$  SEM, after the number of experiments indicated in parentheses.

Compound	EC50
Nabilone	3.90 $\pm$ 1.1 (3)
Anandamide	no effect (3)

The experimental data reported above prove a precise structure-activity correlation in the selective activation of CB2 peripheral receptor, which mediates the non-psychoactive effects of cannabinoids.  
15 More precisely, the molecules having a saturated acyclic chain are able to bind and, above all, to functionally activate CB2 peripheral receptor to a significant extent. To the contrary, compounds with more than one double bond in the acyl chain, though having affinity to CB2 receptor, are not able to activate said receptor functionally, thus  
20 acting as partial competitive receptor antagonists.

- 48 -

Furthermore, the aforementioned experimental results demonstrate that mast cells express the cannabinoid peripheral receptor and that the amidic derivatives according to the present invention behave as competitive agonists of CB2 receptor with an affinity that is higher  
5 than, or in any case comparable with the one of natural and synthetic cannabinoids. Moreover, they functionally activate said receptor.

Importantly, the Applicant has found that also non-immune cells functionally express the cannabinoid peripheral receptor CB2, which for example was observed in cerebellar granule cells, cerebellum,  
10 heart and lung.

Said results are extremely interesting from a therapeutic point of view: in fact, the amides of the invention are suitable for the treatment of all diseases for which cannabinoids are notoriously efficacious, acting only on CB2 peripheral receptor and excluding the  
15 activity mediated by CB1 central receptor, responsible for untoward effects.

It is also very important the fact that the amidic derivatives according to the invention can directly or indirectly regulate the defence response to various noxae involving both cell types belonging  
20 to the immune lineage and neurons or cells having different physiological function, i.e. heart and lung, by modulating mast cell degranulation through the selective activation of CB2 receptor. The importance of mast cells in inflammatory processes connected with several acute and chronic pathologies is well known in the state of  
25 the art (Otten U. et al., Nerve Growth Factor induces growth and differentiation of human B lymphocytes, PNAS, 86:10059-10063, 1989; Marshall J. S. et al., 1989, The J. of Immunology, 144:1866-92; B:

- 42 -

Henderson and S. Blake, Therapeutic potential of cytokine manipulation, TIPS, 13,145-152, 1992; M.C.Morganti-Kossmann et al., Cytokines and neuropathology, TIPS, 13, 286-290, 1992; A.S. Fauci, Science, 262, 1011- 1018, 1993; E.A. Formukong et al., Analgesic and  
5 Antiinflammatory Activity of Constituents of Cannabis Sativa L., Inflammation, 12, 4:361- 371, 1988; W.D. Lyman, Drugs of Abuse and Experimental Autoimmune Diseases, Exp. Med. Biology, 288:81-82, 1991; G.H. Gibbons et al., The Emerging Concept of Vascular Remodeling, Mechanisms of Disease, 330, 20: 1431-1438, 1994; P.T. Kovanen, The  
10 mast cell, a potential link between inflammation and cellular cholesterol deposition in atherogenesis, European Heart Journal, 14 suppl. K: 105-117, 1993).

In other words, thanks to their specific characteristics, the amidic derivatives of the invention are able to selectively bind and to  
15 functionally activate CB2 cannabinoid peripheral receptor. Therefore, they represent an important therapeutic tool both for the treatment of pathologies connected with the modulation of CB2 cannabinoid peripheral receptor and for the treatment of the pathologies deriving, for example, from mast cell degranulation influenced by CB2  
20 receptor.

Therefore, the therapeutic use of said compounds is particularly useful in the treatment of diseases connected with an anomalous modulation of CB2 peripheral receptor, or that benefit of the activation of said receptor with consequent negative modulation of  
25 cytotoxic or proinflammatory phenomena, connected with cytokine, neurokinin or enzyme release, or with second messenger activation, such as:

- 50 -

- pathologies connected with the immune system alteration;
  - pathologies with etiologic autoimmune component, such as multiple sclerosis, lateral amyotrophic sclerosis, and the associated muscular spasm;
- 5 - articular acute and chronic inflammatory pathologies, also on autoimmune basis, such as rheumatoid arthritis;
- pathologies caused by biological agents such as viral (HIV) and bacterial encephalitic meningitis, bacterial meningitis, meningitis by cytomegalovirus and AIDS-dementia complex;
- 10 - chronic neurodegenerative pathologies, such as senile dementia, Alzheimer's and Parkinson's diseases, and the associated cachectic symptomatology;
- pathologies with nociception alteration;
  - pathologies associated with EAA excitotoxicity such as cerebral
- 15 ictus, TIA, cerebral and spinal cord trauma, epilepsy, migraine and coreia;
- cardiovascular pathologies associated with vascular remodelling i.e. restenosis after angioplasty including stent application, atherosclerosis and heart attack;
- 20 - pathologies involving pressure alterations (hypertension) at cardiovascular, pulmonary and ocular levels, i.e. glaucoma;
- chronic airway obstruction, including asthma;
  - nausea, also of hyatrogenic nature.

In view of their effects, the amidic derivatives according to the

25 present invention are suitable for the treatment of human and animal diseases.

For all aforesaid diseases, the systemic administration of the claimed

- 51 -

compounds by the oral or parenteral or topical or transdermic ways may be envisaged.

The therapeutically effective dose varies depending on the way of administration, as well as on the disease seriousness; it also varies  
5 depending on the patient's age, weight and general health conditions. In any case, acceptable therapeutic doses may range from 0.1 to 20 mg/kg/die, over variable periods, in any case for at least 30 days.

The pharmaceutical compositions containing as the active principles the amides according to the present invention are inclusive of all  
10 formulations containing pharmaceutically acceptable excipients, that are suitable for the administration of the active ingredients in the forms best suited to the disease to be treated and, in any case, rendering the active ingredient as bioavailable as possible.

In particular, solutions or suspensions for general i.v., s.c. and  
15 i.m. administration, solutions for ophthalmic treatment in the form of eyewash, solid or semisolid formulations in the form of inserts, gels and ointments are to be envisaged. As concerns oral formulations, granular powders, tablets, pills and capsules will be preferred.

As concerns dermic and transdermic administration, creams, ointments,  
20 gels and plasters, where the active ingredient may be included in slow-releasing microspheres, will be preferred.

#### EXAMPLE 19

##### Vials for injection

Every vial contains:

25	Compound of Example 8	20 mg
	Mannitol	12 mg
	Sodium metasulphite	1.3 mg

- 52 -

Benzyl alcohol	80 mg
Propylene glycol	400 mg
Sodium hydroxide	q.s. to pH 7
Water for injectable formulations	q.s. to 2 ml

## 5 EXAMPLE 20

## Tablets

Every tablet contains:

Compound of Example 1	50 mg
Dibasic dihydrated calcium phosphate	135.2 mg
10 Microgranular cellulose	36 mg
Maize starch	7.2 mg
Magnesium stearate	1.8 mg
Hydrogenated vegetable oil	1.2 mg
Precipitated silica	0.6 mg
15 Hydroxypropylene ethylcellulose	4.7 mg
Titanium dioxide	0.3 mg

## EXAMPLE 21

## Eyewash

Every bottle contains:

20 Compound as per Example 7	25 mg
Borax	15 mg
Boric acid	75 mg
Polysorbate 80	15 mg
Lactose	80 mg
25 Phenol	3.9 mg
Disodium edetate	5 mg
Water for injectable formulation	q.s. to 5 ml

- 53 -

**EXAMPLE 22****Soft capsules**

Every capsule contains:

Compound of Example 16	100	mg
5 Excipient: peanut oil O.P.	100	mg

Composition of the capsule:

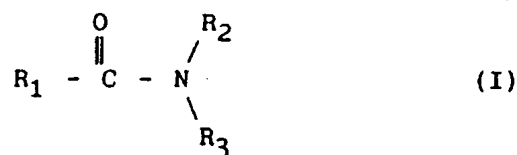
gelatin O.P., glycerin O.P., natural dye E12



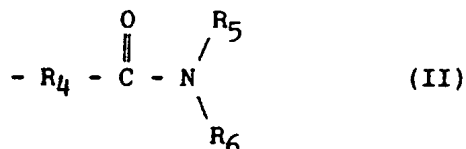
- 54 -

## CLAIMS

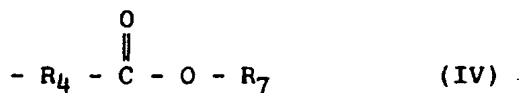
- 1 1. Amide of formula (I):



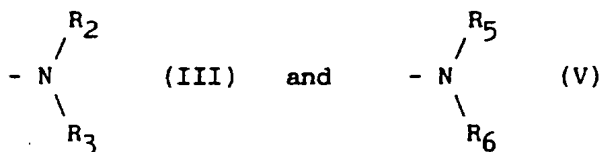
- 2 wherein  $\text{R}_1$  can be:
- 3 1) a linear or branched hydrocarbon radical having from 9 to 23 carbon
- 4 atoms, saturated or presentig one double bond, optionally substituted
- 5 with one or more -OH groups;
- 6 2) a group of formula (II):



- 7 wherein  $\text{R}_4$  is a linear hydrocarbon radical, saturated or containing
- 8 one double bond, comprising from 8 to 22 carbon atoms, optionally
- 9 substituted with one or more linear or branched alkyl groups  $\text{C}_1\text{-C}_8$
- 10 and/or with one or more -OH groups;
- 11 3) a group of formula (IV):



- 12 wherein  $\text{R}_4$  has the meanings decribed above and  $\text{R}_7 = \text{-H}$  or a linear or
- 13 branched alkyl group, comprising from 1 to 20 carbon atoms;
- 14 the residues od formulae:



- 15 where  $\text{R}_2$  and  $\text{R}_3$  are equal or different from  $\text{R}_5$  and  $\text{R}_6$ , are chosen in

- 55 -

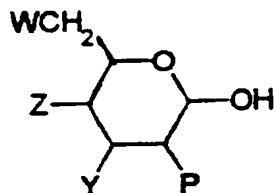
16 one of the following classes:

17 A) a residue of an optically active or non active aminoacid, wherein  
 18  $R_2$  and  $R_5$ , together with the nitrogen atom to which are bound, form an  
 19  $\alpha$ -aminoacid, optionally presenting an aliphatic or arylaliphatic  
 20 lateral chain optionally substituted with  $-\text{OH}$ ,  $-\text{OPO}_3\text{H}_2$ ,  $-\text{O-PO}_2\text{H-O-}$   
 21  $\text{CH}_2\text{-CH(OH)-CH}_2\text{-OH}$ ,  $-\text{SH}$ ,  $-\text{S-CH}_3$ ; or  $R_2$  and  $R_5$ , together with the  
 22 nitrogen atom to which are bound, a  $\beta$ - or  $\gamma$ -aminoacid presenting an  
 23 aliphatic or arylaliphatic lateral chain; the acid group of such  
 24 aminoacid being optionally methylated or ethylated;

25  $R_3$  and  $R_6$  are  $-\text{H}$  or  $-\text{CH}_3$ ;

26 when  $R_1$  belongs to class (1) and  $R_2$  forms, together with the N atom to  
 27 which it is bound, the aminoacylic residue of alanine,  $\beta$ -alanine or  
 28 glycine,  $R_3$  is different from  $-\text{CH}_3$ ;

29 B) a residue of a glycosamine having formula:



30 wherein one of the groups P, Y and Z is  $-\text{N-CO-R}_1$  and the  
 $\text{R}_3$

31 other groups can be  $-\text{H}$  or  $-\text{OH}$ ,  $R_1$  being as above described and  $R_3 = -\text{H}$   
 32 or  $-\text{CH}_3$ ;

33 the residues  $R_2$ ,  $R_3$ ,  $R_5$  and/or  $R_6$  of formulae (III) and (V) being  
 34 optionally substituted with at least a pharmaceutically acceptable  
 35 radical capable of increasing the solubility of the amide of formula  
 36 (I).

1 2. The amide according to claim 1, characterised in that, when  $R_1$

- 56 -

2 belongs to class (1). it is a radical having from 11 to 17 carbon  
3 atoms.

1 3. The amide according to claim 1, characterised in that, when  $R_1$   
2 belongs to class (2),  $R_4$  is a radical containing from 10 to 16 carbon  
3 atoms.

1 4. The amide according to claim 1, characterised in that the residues  
2  $R_2$ ,  $R_3$ ,  $R_5$  and/or  $R_6$  in formulae (III) and (V) are substituted with  
3 at least a pharmaceutically acceptable radical selected from the group  
4 consisting of acyls, emiacyls of bicarboxylic acids, dialkylamino-  
5 acyls, alkyl-sulphonates, alkyls, O-phosphates and O-sulphates.

1 5. The amide according to claim 4, characterised in that said acyl is  
2 acetyl or benzoyl.

1 6. The amide according to claim 4, characterised in that said emiacyl  
2 of a bycarboxylic acid is the emiacyl of succinic or glutaric acid.

1 7. The amide according to claim 4, characterised in that said alkyl-  
2 sulphonate is metansulphonate or p-toluen-sulphonate.

1 8. The amide according to claim 4, characterised in that said alkyl is  
2 methyl or ethyl.

1 9. The amide according to claim 1, characterised in that, when  $R_1$   
2 belongs to class (1), it forms, together with the adjacent carbonyls,  
3 the acyl group of a monocarboxylic acid selected from the group  
4 consisting of lauric acid, myristic acid, palmitic acid, stearic acid,  
5 palmitoleic acid, oleic acid and  $\omega$ -hydroxy-palmitic acid.

1 10. The amide according to claim 1, characterised in that, when  $R_1$   
2 belongs to classes (2) and (3),  $R_4$  forms with the two adjacent  
3 carbonyls the acyl group of traumatic acid.

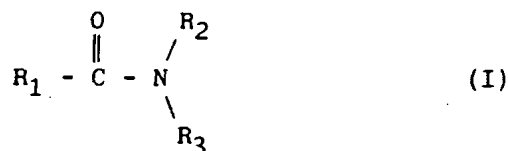
1 11. The amide according to claim 1, characterised in that, when the

- 57 -

2 residues of formula (III) and (V) belongs to class (A), they are the  
 3 residue of an aminoacid selected from the group consisting of glycine,  
 4  $\beta$ -alanine,  $\gamma$ -amino-butyric acid, serine and cysteine.

1 12. The amide according to claim 1, characterised in that, when the  
 2 residues of formula (III) and (V) belongs to class (B), said  
 3 glycosamine is selected from the group consisting of D-Glucosamine,  
 4 L-Acosamine, D- Mannosamine and D-Galactosamine.

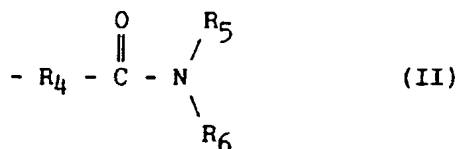
1 13. Process for the preparation of amides of formula (I):



2 wherein  $\text{R}_1$  can be:

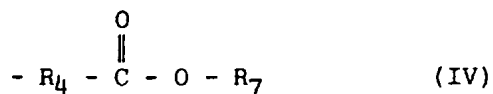
3 1) a linear or branched hydrocarbon radical having from 9 to 23 carbon  
 4 atoms, saturated or presentig one double bond, optionally substituted  
 5 with one or more -OH groups;

6 2) a group of formula (II):



7 wherein  $\text{R}_4$  is a linear hydrocarbon radical, saturated or containing  
 8 one double bond, comprising from 8 to 22 carbon atoms, optionally  
 9 substituted with one or more linear or branched alkyl groups  $\text{C}_1\text{-C}_8$   
 10 and/or with one or more -OH groups;

11 3) a group of formula (IV):

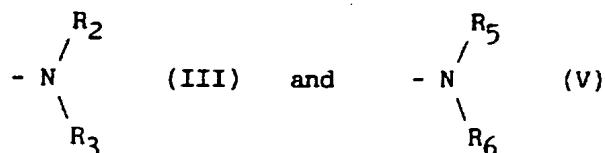


12 wherein  $\text{R}_4$  has the meanings decribed above and  $\text{R}_7 = \text{-H}$  or a linear or

- 58 -

13 branched alkyl group, comprising from 1 to 20 carbon atoms;

14 the residues of formulae



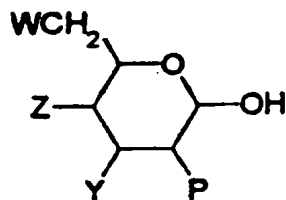
15 where  $\text{R}_2$  and  $\text{R}_3$  are equal or different from  $\text{R}_5$  and  $\text{R}_6$ , are chosen in  
16 one of the following classes:

17 A) a residue of an optically active or non active aminoacid, wherein  
18  $\text{R}_2$  and  $\text{R}_5$ , together with the nitrogen atom to which are bound, form an  
19  $\alpha$ -aminoacid, optionally presenting an aliphatic or arylaliphatic  
20 lateral chain optionally substituted with  $-\text{OH}$ ,  $-\text{OPO}_3\text{H}_2$ ,  $-\text{O}-\text{PO}_2\text{H}-\text{O}-$   
21  $\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2-\text{OH}$ ,  $-\text{SH}$ ,  $-\text{S}-\text{CH}_3$ ; or  $\text{R}_2$  and  $\text{R}_5$  form, together with the  
22 nitrogen atom to which are bound, a  $\beta$ - or  $\gamma$ -aminoacid presenting an  
23 aliphatic or arylaliphatic lateral chain; the acid group of such  
24 aminoacid being optionally methylated or ethylated;

25  $\text{R}_3$  and  $\text{R}_6$  are  $-\text{H}$  or  $-\text{CH}_3$ ;

26 when  $\text{R}_1$  belongs to class (1) and  $\text{R}_2$  forms, together with the N atom to  
27 which it is bound, the aminoacyclic residue of alanine,  $\beta$ -alanine or  
28 glycine,  $\text{R}_3$  is different from  $-\text{CH}_3$ ;

29 B) a residue of a glycosamine having formula:



30 wherein one of the groups P, Y and Z is  $-\text{N}-\text{CO}-\text{R}_1$  and the  

$$\begin{array}{c} | \\ \text{R}_3 \end{array}$$

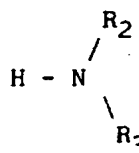
31 other groups can be  $-\text{H}$  or  $-\text{OH}$ ;  $\text{R}_1$  being as above described and  $\text{R}_3 = -\text{H}$

- 59 -

32 or -CH<sub>3</sub>.

33 the residues R<sub>2</sub>, R<sub>3</sub>, R<sub>5</sub> and/or R<sub>6</sub> of formulae (III) and (V) being  
 34 optionally substituted with at least a pharmaceutically acceptable  
 35 radical capable of increasing the solubility of the amide of formula  
 36 (I);

37 comprising the reaction of an activated form of the acid R<sub>1</sub>-COOH, of  
 38 formula R<sub>1</sub>-CO-X, with an amine of formula



39 in aqueous or organic solvents in the presence of a base, under  
 40 stirring at a temperature ranging from -20° to - 60°C, for a period  
 41 ranging from 10 minutes to 24 hours; the optional acidification of the  
 42 reaction medium; the isolation and the purification by conventional  
 43 techniques.

1 14. The process according to claim 13, characterised in that said  
 2 temperature is comprised between -10° and 20°C.

1 15. The process according to claim 13, characterised in that the  
 2 reaction time is comprised between 1 hour and 3 hours.

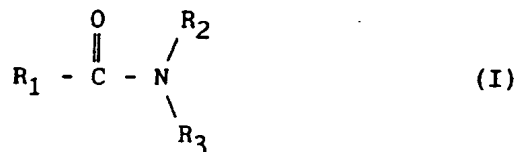
1 16. The process according to claim 13, characterised in that said  
 2 activated form of the acid is selected from the group consisting of  
 3 acyl halide, succinimidylester, acylisourea, p-NO<sub>2</sub>-phenylester and  
 4 methylester.

1 17. The process according to claim 13, characterised in that said base  
 2 is selected from the group consisting of K<sub>2</sub>CO<sub>3</sub>, KOH, Et<sub>3</sub>N and N-  
 3 methyl- morpholine.

1 18. Use in the therapeutic treatment of human and animal pathologies

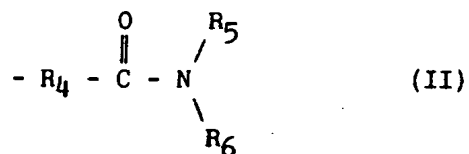
- 60 -

2 connected with the modulation of CB2 peripheral receptor, and with the  
 3 degranulation and/or lysis of immunocompetent cells controlled by CB2  
 4 receptor, of amides of formula (I):

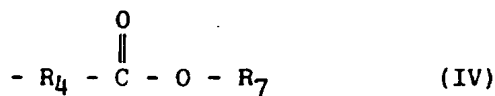


5 wherein  $\text{R}_1$  can be:

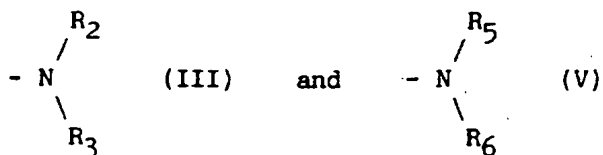
- 6 1) a linear or branched hydrocarbon radical having from 9 to 23 carbon  
 7 atoms, saturated or presenting one double bond, optionally substituted  
 8 with one or more -OH groups;  
 9 2) a group of formula (II):



- 10 wherein  $\text{R}_4$  is a linear hydrocarbon radical, saturated or containing  
 11 one double bond, comprising from 8 to 22 carbon atoms, optionally  
 12 substituted with one or more linear or branched alkyl groups  $\text{C}_1\text{-C}_8$   
 13 and/or with one or more -OH groups;  
 14 3) a group of formula (IV):



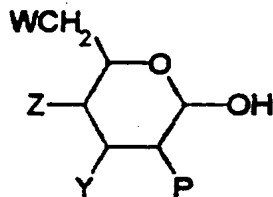
- 15 wherein  $\text{R}_4$  has the meanings described above and  $\text{R}_7 = \text{-H}$  or a linear or  
 16 branched alkyl group, comprising from 1 to 20 carbon atoms;  
 17 the residues of formulae



- 61 -

18 where  $R_2$  and  $R_3$  are equal or different from  $R_5$  and  $R_6$ , are chosen in  
 19 one of the following classes:

- 20 A) a residue of an optically active or non active aminoacid, wherein  
 21  $R_2$  and  $R_5$ , together with the nitrogen atom to which are bound, form an  
 22  $\alpha$ -aminoacid, optionally presenting an aliphatic or arylaliphatic  
 23 lateral chain optionally substituted with  $-\text{OH}$ ,  $-\text{OPO}_3\text{H}_2$ ,  $-\text{O}-\text{PO}_2\text{H}-\text{O}-$   
 24  $\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2-\text{OH}$ ,  $-\text{SH}$ ,  $-\text{S}-\text{CH}_3$ ; or  $R_2$  and  $R_5$  form, together with the  
 25 nitrogen atom to which it is bound, a  $\beta$ - or  $\gamma$ -aminoacid presenting an  
 26 aliphatic or arylaliphatic lateral chain; the acid group of said  
 27 aminoacid being optionally methylated or ethylated;
- 28  $R_3$  and  $R_6$  are  $-\text{H}$  or  $-\text{CH}_3$ ;
- 29 B) a residue of a glycosamine having formula:



30 wherein one of the groups P, Y and Z is  $-\text{N}-\text{CO}-\text{R}_1$  and the  
 $\text{R}_3$

31 other groups can be  $-\text{H}$  or  $-\text{OH}$ ;  $\text{R}_1$  being as above described and  $\text{R}_3 = -\text{H}$   
 32 or  $-\text{CH}_3$ ;

33 the residues  $R_2$ ,  $R_3$ ,  $R_5$  and/or  $R_6$  of formulae (III) and (V) being  
 34 optionally substituted with at least a pharmaceutically acceptable  
 35 radical capable of increasing the solubility of the amide of formula  
 36 (I).

1 19. The use according to claim 18, characterised in that said human  
 2 pathologies are pathologies connected with the immune system  
 3 alteration.



- 62 -

1 20. The use according to claim 18, characterised in that said human  
2 pathologies are pathologies with etiologic autoimmune component,  
3 selected from the group consisting of multiple sclerosis, lateral  
4 amyotrophic sclerosis and the associated muscular spasm.

1 21. The use according to claim 18, characterised in that said human  
2 pathologies are articular acute and chronic inflammatory pathologies.

1 22. The use according to claim 21, characterised in that said chronic  
2 inflammatory pathology is rheumatoid arthritis.

1 23. The use according to claim 18, characterised in that said human  
2 pathologies are pathologies caused by biological agents selected from  
3 the group consisting of viral (HIV) and bacterial encephalitic  
4 meningitis, bacterial meningitis, meningitis by cytomegalovirus and  
5 AIDS-dementia complex.

1 24. The use according to claim 18, characterised in that said human  
2 pathologies are chronic neurodegenerative pathologies selected from  
3 the group consisting of senile dementia, Alzheimer's and Parkinson's  
4 diseases, and the associated cachectic symptomatology.

1 25. The use according to claim 18, characterised in that said human  
2 pathologies are pathologies with nociception alteration.

1 26. The use according to claim 18, characterised in that said human  
2 pathologies are pathologies associated with EAA excitotoxicity,  
3 selected from the group consisting of cerebral ictus, TIA, cerebral  
4 and spinal cord trauma, epilepsy, migraine and corea.

1 27. The use according to claim 18, characterised in that said human  
2 pathologies are cardiovascular pathologies associated with vascular  
3 remodelling, atherosclerosis or heart attack.

1 28. The use according to claim 18, characterised in that said human

- 63 -

2 pathologies are pathologies involving pressure alterations at  
3 cardiovascular, pulmonary or ocular level.

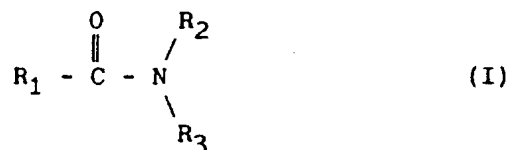
1 29. The use according to claim 28, characterised in that said  
2 pathology is glaucoma.

1 30. The use according to claim 18, characterised in that said human  
2 pathologies are chronic airway obstructions.

1 31. The use according to claim 30, characterised in that said  
2 pathology is asthma.

1 32. The use according to claim 18, characterised in that said human  
2 pathology is nausea.

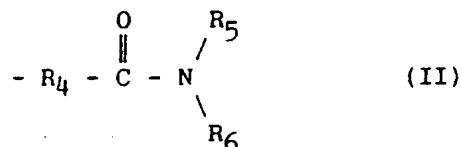
1 33. A pharmaceutical composition containing as the active principle a  
2 therapeutically active amount of amides of formula (I):



3 wherein  $\text{R}_1$  can be:

4 1) a linear or branched hydrocarbon radical having from 9 to 23 carbon  
5 atoms, saturated or presenting one double bond, optionally substituted  
6 with one or more -OH groups;

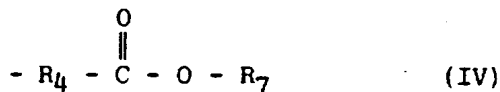
7 2) a group of formula (II):



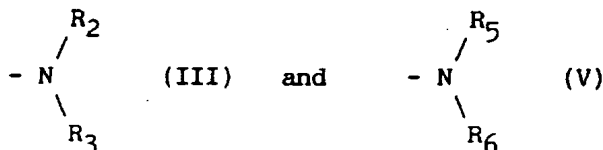
8 wherein  $\text{R}_4$  is a linear hydrocarbon radical, saturated or containing  
9 one double bond, comprising from 8 to 22 carbon atoms, optionally  
10 substituted with one or more linear or branched alkyl groups  $\text{C}_1\text{-C}_8$   
11 and/or with one or more -OH groups;

- 64 -

12 3) a group of formula (IV):



13 wherein  $R_4$  has the meanings described above and  $R_7 = -H$  or a linear or  
 14 branched alkyl group, comprising from 1 to 20 carbon atoms;  
 15 the residues of formulae:

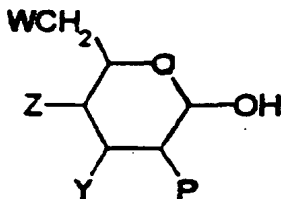


16 where  $R_2$  and  $R_3$  are equal or different from  $R_5$  and  $R_6$ , are chosen in  
 17 one of the following classes:

18 A) a residue of an optically active or non active aminoacid, wherein  
 19  $R_2$  and  $R_3$ , together with the nitrogen atom to which are bound, form an  
 20  $\alpha$ -aminoacid, optionally presenting an aliphatic or arylaliphatic  
 21 lateral chain optionally substituted with  $-OH$ ,  $-OPO_3H_2$ ,  $-O-PO_2H-O-$   
 22  $CH_2-CH(OH)-CH_2-OH$ ,  $-SH$ ,  $-S-CH_3$ ; or  $R_2$  and  $R_5$  form, together with the  
 23 nitrogen atom to which are bound, a  $\beta$ - or  $\gamma$ -aminoacid presenting an  
 24 aliphatic or arylaliphatic lateral chain; the acid group of said  
 25 aminoacid being optionally methylated or ethylated;

26  $R_3$  and  $R_6$  are  $-H$  or  $-CH_3$ ;

27 B) a residue of a glycosamine having formula:



28 wherein one of the groups P, Y and Z is  $-N-CO-R_1$  and the  
 $\quad \quad \quad |$   
 $\quad \quad \quad R_3$

- 65 -

29 other groups can be -H or -OH;  $R_1$  being as above described and  $R_3 = -H$   
30 or  $-CH_3$ ;

31 the residues  $R_2$ ,  $R_3$ ,  $R_5$  and/or  $R_6$  of formulae (III) and (V) being  
32 optionally substituted with at least a pharmaceutically acceptable  
33 radical capable of increasing the solubility of the amide of formula  
34 (I); in combination with pharmaceutically acceptable excipients.

1 34. The pharmaceutical composition according to claim 33, wherein said  
2 active principle is administered in quantities ranging from 0.1 and  
3 20 mg/kg/die.

1 35. The pharmaceutical composition according to claim 33,  
2 characterised in being administered by oral, parenteral, topical or  
3 transdermical route.

1 36. The pharmaceutical composition according to claim 35,  
2 characterised in that said parenteral route is endovenous,  
3 subcutaneous or intramuscular route, and said composition is in the  
4 form of injectable solution or suspension.

1 37. The pharmaceutical composition according to claim 35, characterised  
2 in that said topic route is the ophtalmic route, and said composition  
3 is in the form of eyewash, solid or semi-solid formulation in the form  
4 of insert, gel or ointment.

1 38. The pharmaceutical composition according to claim 35,  
2 characterised in being orally administered in the form of granular  
3 powder, tablets, dragees or capsulas.

1 39. The pharmaceutical composition according to claim 35,  
2 characterised in being dermically or transdermically administered in  
3 the form of cream, ointment, gel or plaster, said active principle  
4 being optionally contained in slow-releasing microspheres.

- 66 -

1 40. The pharmaceutical composition according to claim 33, usefull in  
2 the therapeutic treatment of human and animal pathologies connected  
3 with the modulation of cannabinoids peripheral receptor CB2, or with  
4 the degranulation and/or lysis of immunocompetent cells controlled by  
5 CB2 receptor.

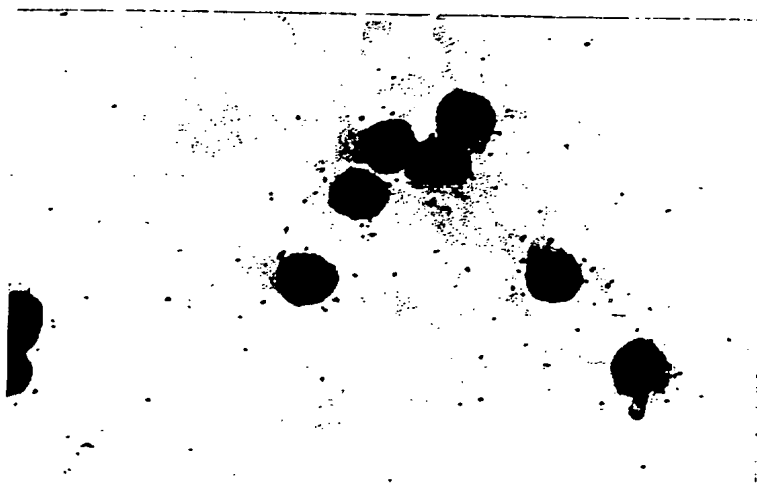
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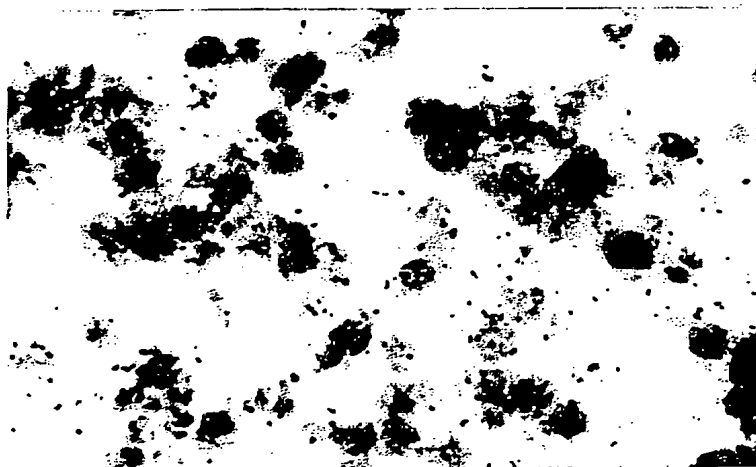


FIG. 1

2/2



A



B

FIG. 2

# INTERNATIONAL SEARCH REPORT

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 6 C07C233/47 C07C323/59 C07H13/06 C07D207/16 A61K31/195 A61K31/22 A61K31/70 A61K31/40	Intern Application No PCT/EP 95/04926	
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07C C07H C07D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 552 405 (LINTEC CORP., JAPAN) 28 July 1993 see page 10, table 2, compounds 3 - 7, 11, 12, 16, 17	1,2,9,11
X	--- CHEMICAL ABSTRACTS, vol. 89, no. 14, 2 October 1978 Columbus, Ohio, US; abstract no. 117553, MORI, MOTOO ET AL: "Stabilized hair dye compositions" XP002001421 see RN 67603-49-0, L-Cysteine, N-(1-oxohexadecyl)- see RN 67603-51-4, L-Cysteine, N-(1-oxo-9-octadecenyl)-, (Z) & JP,A,53 059 041 (KANEBO, LTD., JAPAN) --- -/--	1,2,9,11
<div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.                     </div> <div> <input checked="" type="checkbox"/> Patent family members are listed in annex.                     </div> </div>		
* Special categories of cited documents :		
<div style="display: flex;"> <div style="width: 50%;"> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 50%;"> <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>*&amp;* document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search  <div style="text-align: center; font-size: 1.2em;">24 April 1996</div>		Date of mailing of the international search report  <div style="text-align: center; font-size: 1.2em;">13.05.96</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016		Authorized officer  <div style="text-align: center; font-size: 1.2em;">Seufert, G</div>



## INTERNATIONAL SEARCH REPORT

 Intern. Application No  
 PCT/EP 95/04926

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>J. MED. CHEM. (1977), 20(11), 1362-71 ,            XP002001420            ACTON, EDWARD M. ET AL: "Antitumor            septacidin analogs"            see page 1369, left-hand column, line 47 -            line 52            see page 1369, left-hand column, line 70 -            line 76</p>	1,2,9,11
X	<p>---            CHEMICAL ABSTRACTS, vol. 118, no. 20,            17 May 1993            Columbus, Ohio, US;            abstract no. 194576,            ZHU, JIANGUANG ET AL: "4R0-X collectors            for flotation of calciferous minerals"            XP002001422            see RN 58725-44-3, Butanoic acid,            4-[(1-oxohexadecyl)amino]-            &amp; FEIJINSHUKUANG (1991), (4), 19-22 ,</p>	1,2,9,11
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X	<p>---            CHEMICAL ABSTRACTS, vol. 70, no. 9,            3 March 1969            Columbus, Ohio, US;            abstract no. 38057,            SCIORTINO, T. ET AL: "Antiviral activity.            VIII. Higher N-acyl derivatives of some            amino acids"            XP002001424            see RN 21394-64-9, Serine, N-palmitoyl-,            DL            see RN 16417-38-2, L-Serine,            N-(1-oxohexadecyl)-            see RN 14379-56-7, L-Serine,            (N-1oxododecyl)-            see RN 21394-66-1, DL-Serine,            N-(1-oxododecyl)-            &amp; BOLL. CHIM. FARM. (1968), 107(8),            498-505,</p>	1,2,9,11
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## INTERNATIONAL SEARCH REPORT

 Internatic Application No  
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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US,A,4 999 417 (DOMB, ABRAHAM J.) 12 March 1991 see example 3 and table 2, compound 1 ---	1,3,11
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 82, no. 23, 9 June 1975 Columbus, Ohio, US; abstract no. 156724, KONDO, KIYOSHI ET AL: "N-(.beta.-Hydroxyalkanoyl).alpha.-amino acids and esters" XP002001431 see RN 55713-79-6, L-Serine, N-(3-hydroxy-1- oxotridecyl)- see also RN 55713-85-4, 55713-84-3, 55713-83-2, 55713-82-1,... & JP,A,49 127 918 (SAGAMI CHEMICAL RESEARCH CENTER)	1,2,11
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P,A	--- PROSTAGLANDINS, LEUKOTRIENES ESSENT. FATTY ACIDS (1995), 53(4), 301-8, XP000569157 FONTANA, A. ET AL: "Analysis of anandamide, an endogenous cannabinoid substance, and of other natural N-acylethanolamines" see page 301, left-hand column, line 1 - page 302, left-hand column, line 7 -----	1,18

# INTERNATIONAL SEARCH REPORT

Int. tional application No.

PCT/EP 95/ 04926

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  

Claims searched incompletely: 1-40  
Please see attached sheet ./.
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

Amides of mono and bicarboxylic acids with amino acids or glycosamines, selectively active on the peripheral receptor for cannabinoids and their use in the treatment of pathologies connected with the modulation of said receptor are described. The process for the preparation of said amides and pharmaceutical compositions containing the same are also described.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 95/04926

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